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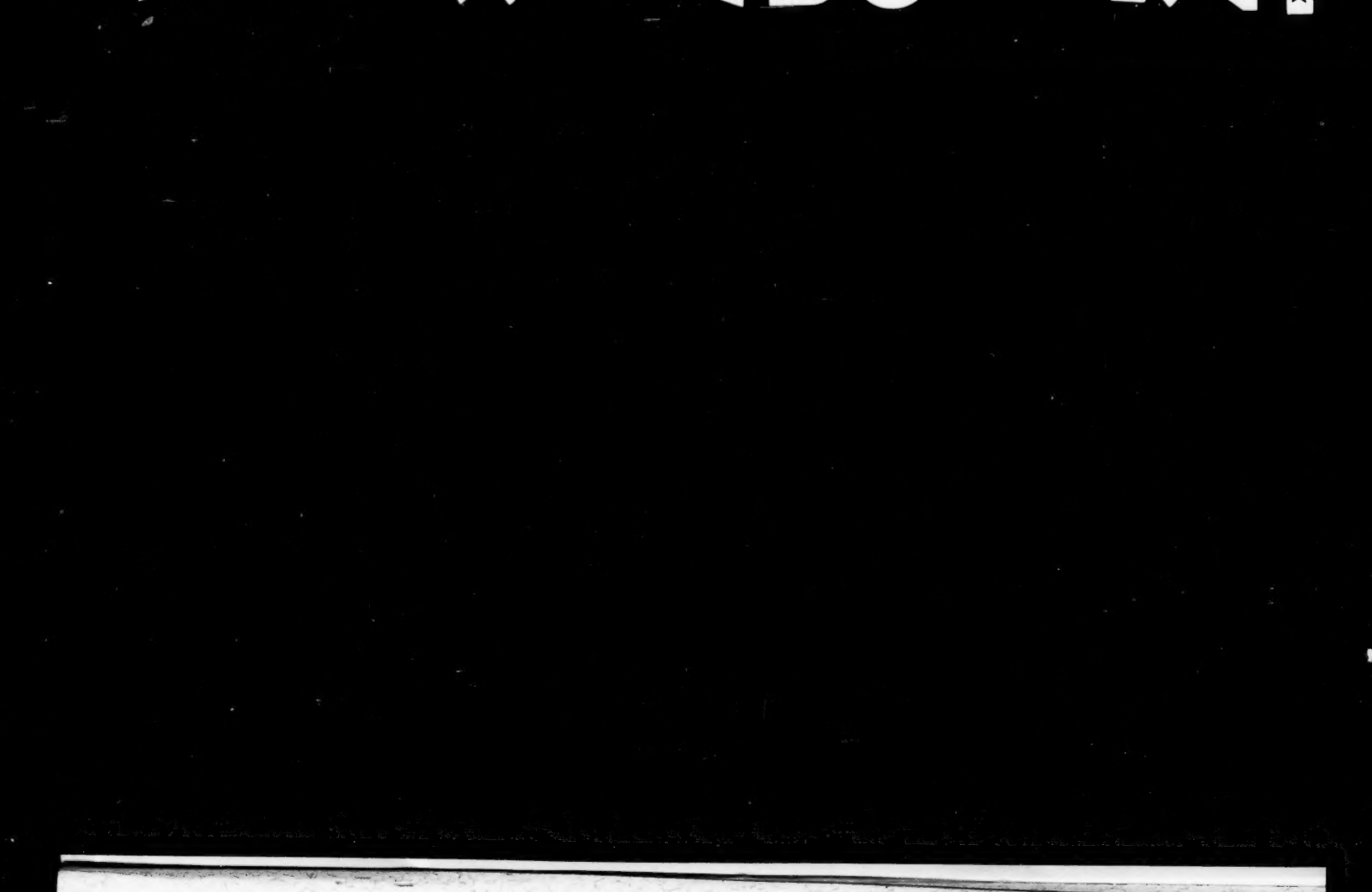

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Contents of previous issues of the JOURNAL OF ANIMAL SCIENCE are indexed in the Agricultural Index which is available in most libraries and in the November issue of each complete volume of the JOURNAL.

THE INFLUENCE OF CERTAIN FACTORS UPON THE DIGESTIBILITY AND INTAKE OF PASTURE HERBAGE BY BEEF STEERS [1]

W. F. Brannon, J. T. Reid and J. I. Miller
Cornell University [2]

RECENTLY there has been a renewed interest in studying the herbage intake of grazing animals. Percival (1950) determined the dry matter consumption of 35 grazing cows by the use of chromium oxide and fecal nitrogen as indicators. Kane, *et al.* (1953) quite accurately measured the herbage intakes of three grazing cows which received 4 lb. of concentrates per head daily. Their estimates were based on a combination of the chromogen and chromium oxide indicator methods. Similar procedures were employed by McCullough (1953) to measure the dry matter intakes of 10 milking cows. These cows received hay, as well as concentrates, in addition to herbage consumed by grazing.

It has been found that the chromogen and chromium oxide indicator methods result in accurate estimates of pasture intake when no supplements are fed (Hardison and Reid, 1953). The present report is a study of pasture herbage consumption by steers allowed to consume concentrates in addition to the herbage. The methods developed by Hardison and Reid (1953) were applied. The objectives of this experiment were to determine: (1) the seasonal variation in the digestibility and intake of pasture herbage when grazed alone and when supplemented by either corn or molasses, (2) the accuracy of the sampling method and other procedures developed by Hardison and Reid for estimating the intake of grazing animals being fed concentrates, (3) the relationship of intake to response of beef steers.

Experimental

For a 30-day preliminary period and a 109-day trial period during the summer of 1952, six beef steers, 12 months old, continuously

[1] From a portion of a thesis presented by W. F. Brannon to the Graduate School, Cornell University, in partial fulfillment of the requirements for the Ph.D. degree, 1953.
[2] Ithaca, New York.

grazed a 9-acre pasture consisting of one-third legumes (white clover, ladino, red clover and alfalfa) and two-thirds grasses (Kentucky bluegrass, timothy and orchard grass). Two steers were allotted to each of the following treatments: (1) pasture herbage alone, (2) cane molasses in addition to pasture herbage, (3) ground yellow corn in addition to pasture herbage. The concentrates were accurately weighed and fed *ad libitum* twice daily to the animals while they were tied in individual stalls for approximately 1½ hours at 6:00 a.m. and 2 hours at 4:00 p.m.

TABLE 1. CHROMIUM OXIDE-CHROMOGEN ESTIMATES OF FECAL OUTPUT AND FEED INTAKE COMPARED WITH MEASURED OUTPUTS AND INTAKES

Dry Matter (lb. per day)

Treatment	Steer Number	Fecal Output		Total Feed Intake	
		Measured [1]	Est.[2]	Measured [3]	Est.[4]
Pasture only	19	4.52	4.46	13.62	13.45
	57	4.19	4.17	12.81	12.75
Pasture plus molasses	153	5.08	5.14	15.22	15.38
	58	4.15	4.10	13.80	13.94
Pasture plus corn	22	4.41	4.44	14.07	14.16
	60	5.61	5.86	18.30	19.09

[1] Total fecal output obtained by use of collection bags.
[2] Estimates of fecal output were calculated from the chromium oxide concentration in composites of 6 a.m. and 4 p.m. "grab" samples.
[3] Intake computed from total fecal collections.
[4] Intake estimated by chromium oxide-chromogen technique.

The herbage intakes of the steers were measured by the use of chromium oxide and chromogen indicators as outlined by Hardison and Reid (1953). Five grams of chromium oxide in a gelatin capsule were given orally to each steer at 7 a.m. each day throughout the grazing season. Beginning 5 days after the first chromium oxide administration, fecal samples were taken directly from the rectum at 6:00 a.m. and 4:00 p.m. daily. These "grab" samples were compounded on an equal weight basis during periods of not less than 4 days and the composites were analyzed for the two indicators.

The summer was divided into 8 periods, the length of the periods varying from 4 to 23 days according to management practices such as clipping and fertilizing of the pasture; environmental conditions such as rainfall and dry, hot weather; and the availability of aftermath.

Results and Discussion

Accuracy of Chromium Oxide-Chromogen Method for Estimating Intakes

The accuracy of the chromium-oxide-chromogen technique for estimating total intakes was determined by comparison with intakes calculated from indigestibility (derived from fecal chromogen concentration) and total collections of feces during a 4-day period. Table 1 shows a very close agreement between the estimated values and those

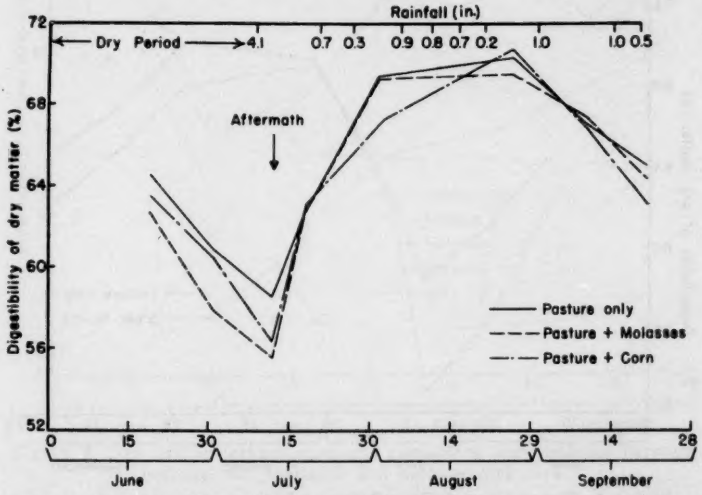

Figure 1. Digestibility of herbage dry matter by steers consuming pasture herbage unsupplemented and supplemented by corn and molasses.

derived from the total collections. The greatest deviation occurred with steer 60. This error is about 4 percent, which is no greater than one might expect with biological materials. At the time this comparison was made, steers 60 and 22 were receiving over two-thirds of the dry matter of their rations as corn, and steers 58 and 153 were receiving about one-seventh of the dry matter of their rations as molasses.

Digestibility of Pasture Herbage

The digestibility of the herbage dry matter, measured continuously, showed definite trends as the season progressed. These trends are presented in figure 1. Digestibility declined during the early summer

months as the forage became more mature. It reached a low during early July at the end of a prolonged period of very dry, hot weather. A good soaking rain then started aftermath growth which is reflected in the upward trend of digestibility. Digestibility consistently increased as aftermath became more plentiful. This undoubtedly was influenced by regular rainfalls. Finally, a peak was reached during the last of August when excellent aftermath existed in abundance. This peak was higher than the peak of herbage digestibility of mid-June. However, had the

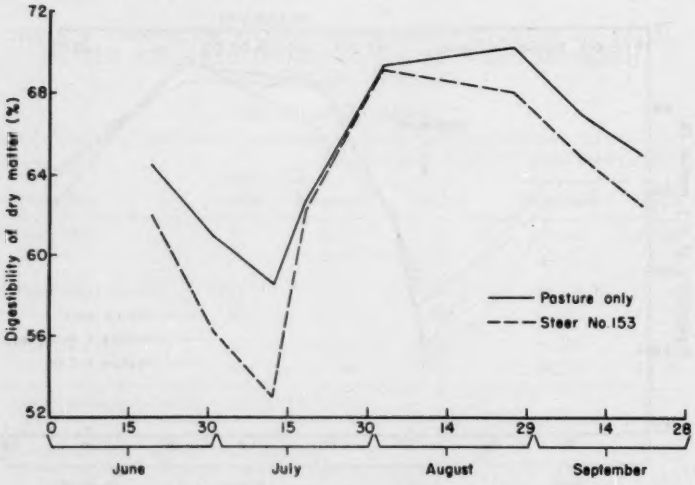

Figure 2. Comparison of digestibility of herbage dry matter by grazing steers not receiving supplements with that by a steer fed a considerable amount of molasses.

digestibility of early spring herbage been determined, it probably would have been higher than that of the aftermath. After reaching this peak, digestibility decreased as the pasture herbage matured in the fall.

In addition to the environmental effects, the supplemental feeds seemed to exert an influence on herbage digestibility. Digestibility of herbage appeared to be depressed slightly by the feeding of corn but this effect may not be significant. However, as shown in figure 2, molasses markedly depressed the digestibility of herbage dry matter, particularly during periods when relatively large amounts (5 lb. per day) of molasses were consumed.

Feed Intakes

The intake of herbage paralleled the pattern of herbage digestibility. The amounts of dry matter consumed as herbage, corn and molasses are shown in figure 3. The intakes of pasture herbage were relatively

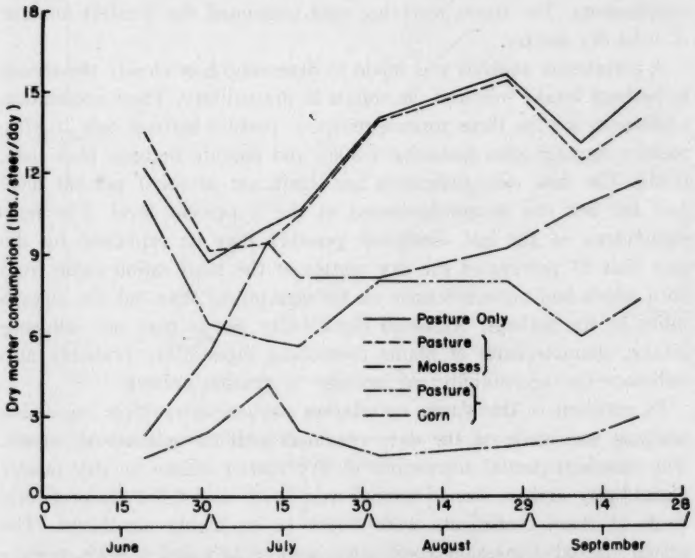

Figure 3. Dry matter intakes of steers consuming only pasture herbage, herbage and molasses and herbage and corn.

high in the spring, gradually dropping to a low in early July when the quantity of available herbage was rather limited due to the clipping and removal on July 8 of the excess early growth, and to dry, hot weather. When the aftermath growth appeared, intakes increased, reaching the highest level during the last of August when there was an abundance of excellent second growth. During the first week of September, herbage intakes of the steers decreased. This was probably due to interference from the fecal collection bags, since intakes increased following their removal.

During early- to mid-July, the supplemental feed intakes increased soon after the digestibility and intakes of herbage decreased (figure 3). This may have been due to the shortage of herbage, to its decreased palatability, or to the exceptionally hot weather limiting the steers'

grazing time. For the corn-fed steers, large intakes of corn were accompanied by a considerable reduction in the intakes of herbage, whereas the herbage intakes of the molasses-fed steers were not greatly affected by the small intakes of molasses. The steers fed supplements consumed more total dry matter daily, as shown in figure 3, than the steers not fed supplements. The steers receiving corn consumed the greatest amount of total dry matter.

A correlation analysis was made to determine how closely the trends in herbage intake followed the trends in digestibility. These correlation coefficients for the three treatments were: pasture herbage only, 0.820; pasture herbage plus molasses, 0.806; and pasture herbage plus corn, 0.253. The first two coefficients are significant at the 1 percent level and the last one is nonsignificant at the 5 percent level. The nonsignificance of the last coefficient possibly may be explained by the fact that 52 percent of the dry matter of the total ration came from corn which had more influence on herbage intake than did the digestibility of the herbage. Although digestibility *per se* may not influence intake, characteristics of plants controlling digestibility probably also influence the acceptability of herbage to grazing animals.

In addition to the simple correlation analysis, a multiple regression analysis was made of the data obtained with the pasture-fed steers. The standard partial regressions of dry matter intake on dry matter digestibility and on size of animal were 0.485 and 0.561, respectively. Both of these coefficients were found to be highly significant. The actual partial regression coefficients were 0.340 and 0.0249, respectively. Therefore, it appears in this study that size of the animal, as well as the digestibility (or properties of herbage determining digestibility) of the herbage dry matter, is an important factor influencing the consumption of pasture dry matter by grazing steers.

Relation of Intake to Response

The responses of the steers were measured by periods as total gains in live weight. These responses and the cumulative digestible dry matter intakes are recorded in figure 4. The steers increased in live weight as the total intakes of digestible dry matter became larger, suggesting that the different rations were supplying more nutrients than necessary for maintenance. The corn-fed steers consumed the largest amounts of feed and made the greatest gains. With respect to the degree of fatness, this pair of steers ranked first, the molasses-fed steers next, as would be expected, and the pasture-only steers last.

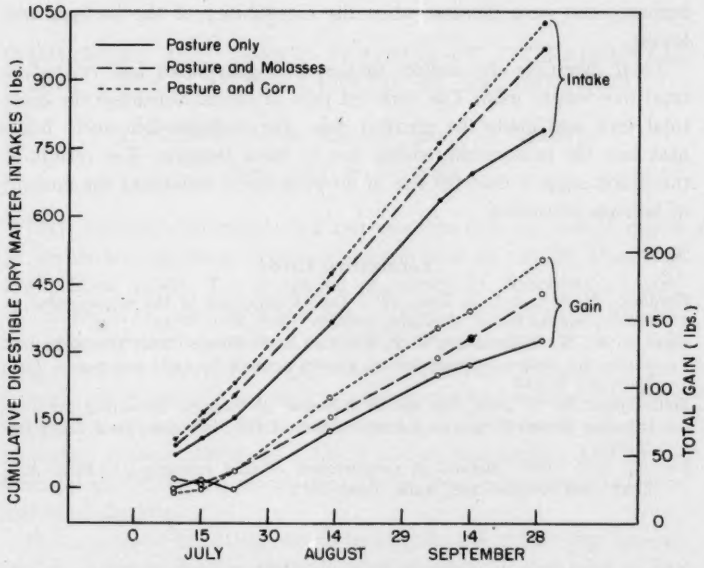

Figure 4. Cumulative digestible dry matter intakes and total gains in body weight.

Summary

During the summer of 1952 the digestibility and intakes of pasture herbage by six beef steers were determined by the use of chromogen and chromium oxide indicators. All steers continuously grazed a 9-acre pasture. Two of the steers obtained their entire ration from pasture herbage, while two others received cane molasses and two received ground, yellow corn in addition to the herbage. Live weight gain records were kept on these animals.

The accuracy of the chromogen-chromium oxide method for measuring dry matter intakes was tested by comparison of intakes so determined with intakes determined from total fecal collections. The method gave highly satisfactory results.

The digestibility of the pasture forage dropped as the forage matured and rose when aftermath growth appeared. There was an indication that molasses depressed the digestibility of consumed herbage. The trend of herbage intakes paralleled the pattern of digestibility. The intakes of supplemental feeds varied inversely with the intakes of

herbage, and were greatest when the digestibility of the herbage was lowest.

Total digestible dry matter intakes were calculated and related to total live weight gain. The corn-fed pair of steers consumed the most total feed and made the greatest gain, the molasses-fed steers being next and the pasture-only steers last in these respects. The results of this study suggest that the size of growing steers influences the amount of herbage consumed.

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INDIVIDUAL AND GROUP FEEDING OF EXPERIMENTAL BEEF CATTLE AS INFLUENCED BY HAY MATURITY[1]

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University of Nevada[2]

THE value of individual feed consumption data in animal experimentation has long been recognized (Carroll, 1930; Crampton, 1930; Lush, 1930). The increasing popularity of "efficiency of gain" or "economy of gain" as a selection criterion for beef cattle breeding and improvement has resulted in the individual feeding of many bulls and, to a lesser extent, heifers. Virtually all of these animals and their progeny are eventually required to perform under conditions of group feeding. A substantial difference in performance between group and individual feeding conditions could result in considerable bias in the practical application of information derived from experiments involving individual feeding.

The purpose of this study was to investigate the relationship between rate of gain and feeding methods as influenced by maturity of hay fed wintering beef cattle.

Materials and Methods

The data consist of weight gains for wintering weanling and long yearling range Hereford steers from 1946 to 1949, and weanling heifers during the winter of 1952–53. The animals were fed in pens at the Knoll Creek Experimental Range Station in northeastern Elko County. The pens are 16 feet wide and 32 feet long and constructed of poles and cable. They are arranged in three rows of twelve pens each. An open shed, running water and mangers adequate for a continuous hay supply were available in each pen.

Twenty-eight weanling steers were fed during the winter of 1946–47. Fourteen were fed early-cut native mountain meadow hay and 14 were fed hay cut later. Within each group eight steers were fed in individual pens and six in pairs. These animals were ranged the following summer, run on aftermath in the fall, penned and fed the second

[1] This work is in cooperation with the Animal and Poultry Husbandry Research Branch, A.R.S., U.S.D.A., under Western Regional Project W-1 on Beef Cattle Breeding Research. It was also supported by a grant from the Sears-Roebuck Foundation.

[2] Department of Animal Husbandry, Reno, Nevada. The authors are indebted to Dr. W. C. Rollins of the University of California for assistance with the statistical analyses.

winter in the same manner as previously. The method of allotment for the second winter may have introduced a bias, particularly if a residual effect from the first year existed. Differences in body weight among the groups were of about the same magnitude at the beginning of the feeding period each winter. This resulted from a negative correlation between gains made during winter feeding and on summer range (Kidwell, 1954). It was assumed, therefore, that such a bias was negligible in these data.

A group of 30 weanling steers was fed in the same manner during the winter of 1948–49. Fifteen were fed early-cut hay and 15 late-cut hay. Within each group nine were fed as individuals, and six in pairs. The data for the years 1946–49 were analyzed by the method of analyses

TABLE 1. AVERAGE DAILY GAIN IN POUNDS OF STEERS AND HEIFERS FED EARLY AND LATE CUT HAY AS INDIVIDUALS AND IN GROUPS

	early cut hay		late cut hay	
Experiment	group	individual	group	individual
Steers	1.01	0.67	0.34	0.34
Heifers	0.37	0.00	—0.24	—0.23

of variance for a 2 × 2 table with disproportionate subclass numbers in the presence of interaction (Snedecor, 1946).

Twenty-four weanling heifers were individually and group-fed early and late-cut hay during the winter of 1952–53. A 2 × 2 factorial design was employed. Each group consisted of six animals as contrasted to the pairs of the preceding trials. In all trials animals were allotted to the experimental treatments at random according to weight.

Results and Discussion

The average daily gain made by the steers (1946 to 49) is summarized in table 1. The difference in average daily gain between the groups receiving early and late-cut hay was 0.48 pounds. Steers fed in pairs averaged 0.20 pound more gain per day than those fed individually. These differences and the interaction are significant at the 1 percent level (table 2). A supplemental analysis indicated that there were no differences due to effects of different years.

The results of the experiment with weanling heifers (table 1) are in close agreement with those of the previous trials. The difference in average daily gain between those fed early and late-cut hay was 0.42

pound. Heifers fed as a group averaged 0.18 pound greater daily gain than those fed individually. The difference between the gains of heifers fed early and late-cut hay was significant at the 1 percent level. The difference in gain between those fed as a group and as individuals and the interaction were significant at the 5 percent level.

It should be noted that the analysis and tests of significance employed do not permit generalizations beyond the conditions of these experiments. The data indicate that under these conditions beef cattle wintering on a higher quality hay will make significantly greater gains if fed in pairs or groups rather than as individuals. When fed a poor quality hay, the method of feeding makes little difference in gain. The results are significant in feedlot "efficiency" or "economy" and "rate of gain"

TABLE 2. ANALYSIS OF VARIANCE OF GAINS OF CATTLE FED EARLY AND LATE CUT HAY AS INDIVIDUALS AND IN GROUPS

Comparison	Degrees of Freedom	Mean Square
Steers		
Early vs. late	1	72,199**
Pair vs. individual	1	8,344**
Interaction	1	8,448**
Error	82	690
Heifers		
Early vs. late	1	13,301**
Groups vs. individual	1	2,501*
Interaction	1	2,709*
Error	20	512

* Significant at the 5% level.
** Significant at the 1% level.

testing of range bulls. They are also of significance in nutrition experiments involving different nutritive levels.

It is impossible to account, in quantitative terms, for the observed differences. There are, however, several factors which may have contributed to them. In these trials it was noted that the individual-fed animals were more nervous than those in pairs or groups. In all cases the animals had a clear view into adjacent pens. The greater restlessness of those fed individually may be associated with these results. Individually fed animals did not consume as much feed as the others. Maynard (1951) states, "Certain species, which are fed together in practice, may consume somewhat less when fed individually." Some of those fed individually wasted more hay than those fed in pairs or groups. These factors are at least partially associated with nervousness,

but cause and effect relationships were not determined. The previous history of the cattle may exert an influence. These cattle were considered a sample of the range population in northeastern Elko County. Cattle produced under farm or other conditions might react differently.

Crampton (1929a, 1929b) compared the rate of gain of growing-fattening swine that were individually and group-fed. The individual-fed animals gained more than those fed as a group. Since the pigs were hand fed the more aggressive animals in a group may have consumed a disproportionate amount of the available feed. In the trials reported here, feed was continuously available, which may account for the difference between the two studies.

Kunkel *et al.* (1953) studied the relationship between rates of gain and serum protein bound iodine with group and individually fed Hereford bulls. Although the method of feeding was confounded with separate experiments, it is of interest to note that with apparently comparable animals the group-fed bulls gained 0.2 to 0.3 pound more per day than those fed individually.

Winters and McMahon (1933) compared the rate of gain of individually and group-fed fattening steers. Individually fed steers were run as a group except for the actual feeding period of about an hour and a half twice a day when they were placed in small individual pens. An attempt was made to offer each steer all he would eat at each feeding. In the first trial, ten group-fed Shorthorn steers made an average daily gain of 2.32 pounds as compared with 2.06 pounds for eight fed individually. It was concluded that the herdsman was unable to follow the appetites of the individually fed steers as well as those fed in a group. In a second trial there was no appreciable difference between rate of gain of 14 individually-fed and 12 group-fed Hereford steers. Winters and McMahon concluded that the method of feeding had little effect on rate or economy of gain.

The failure of the results of this study to agree with the conclusions of Winters and McMahon may be accounted for by differences between the experiments. In these trials individually fed animals were maintained in separate pens, allowed feed continuously and fed wintering rations. In Winters and McMahon's study steers were separated only during the limited feeding period, and fed a fattening ration. It is of interest to note that the difference in gain between individual and group-fed steers observed by Kunkel *et al.* and in Winters and McMahon's first trial is of about the same magnitude as those reported here.

Summary and Conclusions

This paper presents a study of comparative weight gains of range Hereford cattle fed individually and in pairs or groups. On the basis of these data, and under the conditions of these trials, it appears reasonable to conclude that when fed a higher quality hay, group or pair-fed animals will gain more than individually fed animals. When fed a low quality hay there is no difference in gain between individually and group or pair-fed animals. At least part of the difference may be due to a greater restlessness, lower feed consumption and greater feed waste noted in the individually fed animals. It is noted that these results may not necessarily be obtained under other conditions.

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THE RELATION OF ORGAN WEIGHTS TO LEAN BODY MASS AND EMPTY BODY WEIGHT IN CATTLE

H. F. KRAYBILL,[1] R. L. HINER AND V. M. FARNWORTH
United States Department of Agriculture[2]

COMPOSITION of the dressed beef carcass and its cuts is being given increased attention by the consuming public. Therefore, the relative weights or proportions of muscle, fat and bone that make up the live meat animal or its carcass is of major importance in the field of animal production. Reliable and easy methods of determining the composition of a dressed carcass have been studied extensively and are still the aim of the research worker. However, the relation of the weight of an individual visceral organ to any of the morphological components of the body has received little emphasis.

Many different methods of estimating body composition have been suggested and investigated. Pitts (1951) in working with guinea pigs has shown that the heart, liver and kidney weights increase in direct proportion to weight of fat-free body. Certain anthropometric measurements for estimating the proportion of fat, lean or bone in the intact or slaughtered animal have been proposed by Black *et al.* (1938); Mason (1951); and McMeekin (1941). Behnke (1953) in searching for some anatomical entity as an index of composition has postulated that weight of liver might be closely related to the lean body mass of the animal. Brodie (1945) has shown a logarithmic relation between the weights of liver, kidney, thyroid, parathyroid and brain to body weight in mammals ranging in size from the rat to the whale. Similarly Christian (1953) reported that the adrenal gland weight follows a definite logarithmic relationship to body weight for a large group of species. Moulton (1922) in presenting data on the ratio of organ weight to empty body weight in cattle showed that the percentage of each visceral organ comprising the empty body decreased during growth. Hiner (1933) in a comprehensive study with swine showed the relationship of gland and internal organ weights to the rate and character of growth. This study showed the correlation between glands and organs but there was no attempt to relate the gland weight to body weight or lean body mass.

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The lean body mass comprising the bone and muscle tissues (non-fat portion) of the body has been shown repeatedly to be constant in composition Murray (1922). Much of the variability in the body composition of mammals including man is accounted for by variation in fat content. Since the fat content of the body roughly represents the difference between the total body weight and lean body mass it is possible to calculate the fat content from the lean body mass. Recently Miller *et al.* (1952) have reported that the lean body mass and body fat can be estimated from basal oxygen consumption and creatinine excretion.

The purpose of this study was first: to investigate and record the relationship, if any, between weights of organs and the lean body mass and the empty body weight of cattle. Second: to test the hypothesis that liver weight is unique as an index of the weight of the lean body mass in cattle.

Material and Methods

The weights of organs from animals used in this study were obtained from several sources. Data reported by Moulton *et al.* (1922) from the Missouri Agricultural Experiment Station, henceforth referred to as Missouri data, and those by Haecker (1920), as well as unpublished data from the Minnesota Agricultural Experiment Station, referred to as Minnesota data, were selected as typical for steers ranging widely in age and weight. The Missouri cattle ranged in age from 90 to 1460 days and in weight from 186 to 1944 lb., whereas the Minnesota cattle ranged in age from 14 to 1001 days and from 74 to 1465 lb. Limiting the weight and age range further, a group of 15 Beltsville Hereford steers was selected for study. Approaching more nearly a weight and age constant population, some data on 40 dual-purpose type Shorthorn steers weighing about 500 lb. and 38 steers and heifers making up pairs of identical twins of different breeds weighing approximately 1000 lb. were also utilized in this investigation.

Data were obtained on the weights of the liver, heart, spleen, pancreas, kidney, thyroid, adrenal, thymus, pituitary and brain for each of the groups of cattle studied. A preliminary statistical treatment of the data revealed that no close relationship existed between the weights of some organs and the lean body mass or the empty body weight. Therefore, attention was directed specifically to a study of the relationships between the lean body mass, empty body weight and the weights of the following visceral organs: liver, kidney, spleen, heart and pancreas.

TABLE 1. RELATIONSHIP BETWEEN WEIGHT OF VARIOUS VISCERAL ORGANS AND WEIGHT OF LEAN BODY MASS FOR CATTLE OF DIFFERENT WEIGHT CLASSES

Organ and Group[1]	Number of Animals	Weight Class	Correlation Coefficient	Regression Equation[3]	Standard error of Estimate[3]
		Lbs.	"r"	(Y=)	
Liver					
A	40	Circ. 500	0.17	153.5+0.005X	10.7
B	38	Circ. 1000	0.61	142.3+0.035X	32.3
C	30	186–1944	0.93	−10.7+0.076X	49.8
D	33	74–1465	0.98	−1.0+0.058X	16.9
E	15	528–1044	0.69	92.6+0.039X	26.9
F	156	74–1944	0.90	−10.7+0.065X	40.9
Heart					
A	40	Circ. 500	0.58	104.5+0.061X	8.8
B	37	Circ. 1000	0.12	279.2+0.014X	38.9
C	30	186–1944	0.96	−3.2+0.224X	34.8
D	33	74–1465	0.97	6.4+0.160X	22.6
E	12	744–1044	0.48	197.0+0.051X	19.4
F	152	74–1944	0.87	26.3+0.153X	46.9
Spleen					
A	40	Circ. 500	0.41	149.5+0.040X	9.8
B	38	Circ. 1000	0.51	224.7+0.094X	35.0
C	30	186–1944	0.95	27.8+0.302X	23.8
D	[2]	[2]	[2]	[2]	[2]
E	13	528–1044	0.51	130.6+0.206X	33.6
Pancreas					
A	40	Circ. 500	0.17	157.1+0.50X	10.6
B	38	Circ. 1000	0.52	213.1+0.203X	34.9
C	30	186–1944	0.88	73.0+0.467X	63.1
D	[2]	[2]	[2]	[2]	[2]
E	[2]	[2]	[2]	[2]	[2]
Kidney					
A	40	Circ. 500	0.27	148.9+0.044X	10.4
B	[2]	[2]	[2]	[2]	[2]
C	30	186–1944	0.94	−77.5+0.463X	43.9
D	[2]	[2]	[2]	[2]	[2]
E	[2]	[2]	[2]	[2]	[2]

[1] These groups described in the test: A, dual-purpose steer calves; B, identical twin steers and heifers; C, Missouri steer data; D, Minnesota steer data; E, Hereford steer data; F, five groups combined. Groups A, B, and E, were from the Agricultural Research Center, Animal Husbandry Division, Bureau of Animal Industry, Beltsville, Maryland.

[2] Data not available for this group.

[3] The Y value (lean-body mass) is expressed in kilograms and the X value in grams.

Since the weight of cattle is extremely variable because of fluctuation in amount of digestive fill, the more constant empty body weight (slaughter weight minus weight of digestive tract contents) was taken as representative of total body weight. The lean body mass calculated for all the cattle was obtained by subtracting the total body fat (carcass fat plus visceral fat) from the empty body weight.

Results

Moulton *et al.* (1922) showed that the ratio of organ weight to empty body weight decreased during increasing age and fatness, but

TABLE 2. A COMPARISON OF THE RELATIONSHIP BETWEEN THE WEIGHTS OF VARIOUS VISCERAL ORGANS AND WEIGHT OF LEAN BODY MASS AND EMPTY BODY WEIGHT FOR 30 STEERS (MISSOURI DATA) [1]

Organ	Organ Weight Compared with Lean Body Mass			Organ Weight Compared with Empty Body Weight		
	Correlation Coefficient	Std. Error of Estimate	Regression Coefficient (b)	Correlation Coefficient	Std. Error of Estimate	Regression Coefficient (b)
Heart gm.	0.96	34.8	0.224	0.98	45.4	0.376
Kidney gm.	0.94	43.9	0.463	0.92	86.4	0.768
Liver gm.	0.93	49.8	0.076	0.93	80.0	0.129
Spleen gm.	0.95	23.8	0.302	0.89	102.2	0.482
Pancreas gm.	0.88	63.1	0.467	0.89	101.1	0.802

[1] Data on weights of organs published by Moulton *et al.* (1922). the data shown on lean body mass were calculated from the basic data provided by Moulton.

their study unfortunately did not reveal what correlation existed between the weight of visceral organs and total body weight or empty body weight or the non-fat portion of the body, the lean body mass. In the statistical evaluation of the data on the Missouri cattle it was found that there were high positive correlations between the weights of liver, heart, kidney, spleen and pancreas and the weight of the lean body mass (table 2). The particularly high coefficients of correlation for the specified relations would indicate that these visceral organs have some usefulness in the estimation of lean body mass.

In selecting the regression relationship of one organ with lean body mass (Missouri data) in preference to the relationship of another organ the coefficients of correlation are of little value because of the small differences between them. The usual tests for significance are

invalidated when the objective is to detect small differences between coefficients of correlation where one part of an animal is correlated with the whole but the regression equation used for predictive purposes is not invalidated. For the Missouri data a measure of the usefulness of the regression equation will be based largely on the standard error of estimate. On this basis and the regression coefficient (table 2) the heart and spleen might have greater predictive value than the liver in the estimation of lean body mass.

The trimmed kidney (devoid of fat) data, not available for all groups of cattle seem to suggest that this organ is perhaps also a very reliable indicator.

Since the Missouri and Minnesota cattle varied widely with respect to age, weight and degree of fatness it seemed essential that the effect of variations in these factors be removed. The Beltsville identical twin steers and heifers and the small group of Hereford steers weighing approximately 1000 lb. provided two groups of cattle that were similar in these respects. A third group on 500 lb. dual-purpose Shorthorn steers while lighter showed little variation in weight, age and degree of fatness. An analysis of data from these three groups of cattle indicated that when variations in these three factors are limited the close association between organ weight and lean body mass is altered (table 1). The Beltsville identical twin cattle and the Hereford steers show a lower correlation than the Missouri and Minnesota cattle. By further restricting the range in age, weight and degree of fatness, as was the case with the 500 lb. dual-purpose Shorthorn steers, the correlation was significantly reduced, as shown in table 1 and figure 1. The standard error of estimate for this group however, is consistently lower than that for the other groups which show much higher correlations.

Further statistical analyses of these data were made to determine the degree of association between the various visceral organs and empty body weight as shown in table 2. The combined data for lean body mass and empty body weight representing all groups of cattle mentioned were analyzed statistically and these results are shown in table 1. The combined data from five groups of cattle showed that the liver and these components of the body were highly correlated. The correlation coefficient for the relation between liver and lean body mass was 0.90 (table 1) and that between liver and empty body weight (not shown in table 1) was approximately the same. In table 2 the correlation coefficients for these relationships applied to the Missouri steers were nearly identical (0.93).

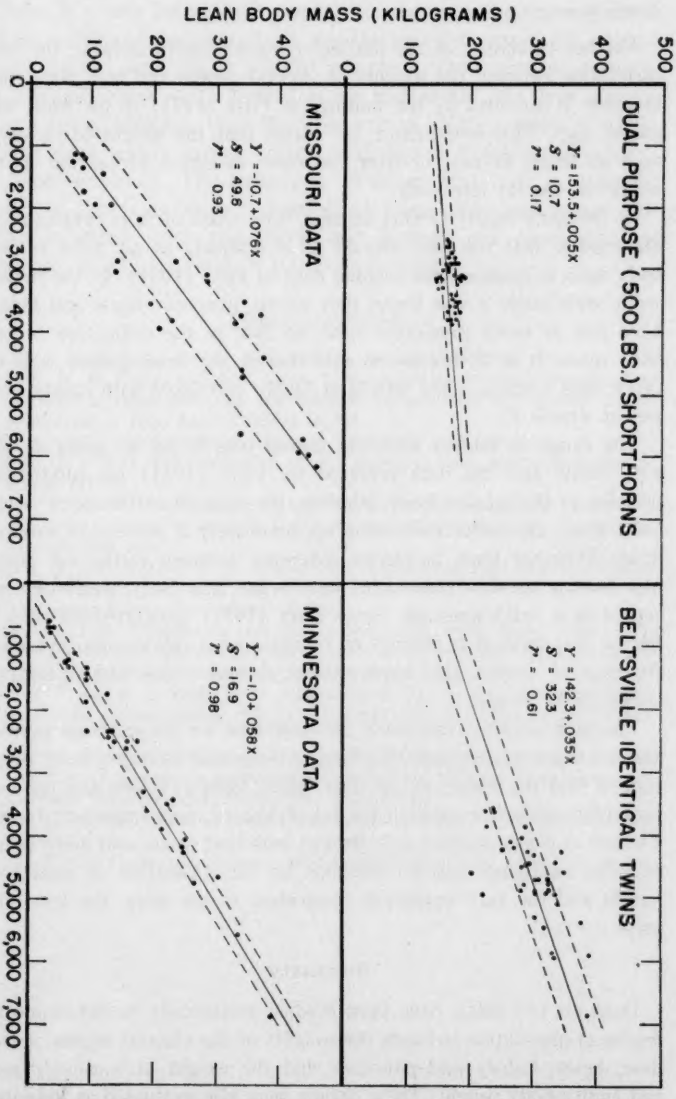

Figure 1. Relation between the weight of the liver and weight of the lean body mass with regression line (——) and lines for the standard error of estimate (– – – –) for four different weight groups of cattle.

Discussion

Further validation of the findings reported herein showing the close correlation between the weights of visceral organs and lean body mass in cattle is provided by the findings of Pitts (1951) in his work with guinea pigs. This investigator has stated that the weight of an organ such as heart, kidney, or liver, increases in direct proportion to the weight of the fat free body.

In Behnke's report (1953) on lean body mass there is evidence that his premise that the liver should serve uniquely as an index of lean body mass is based on the original data of Pitts (1951). In the present study with cattle it was found that spleen, pancreas, heart and kidney have just as much predictive value as liver in the estimation of lean body mass. It is of interest to note that in the investigation with the cattle data visceral organ weight is highly correlated with empty body weight (table 2).

The range in fatness with the guinea pigs is not as great as that with cattle and the data reported by Pitts (1951) on guinea pigs pertains to the fat-free body, whereas, the data on cattle refers to lean body mass, the latter containing approximately 3 percent of essential lipids. Whether there is species difference between cattle and guinea pigs insofar as the relation between organ and body weight is concerned is a valid question. Since Pitts (1951) computed all data to the fat-free basis it is difficult to estimate what relationship existed in the case of guinea pigs between the visceral organ weight and the empty body weight.

The high positive correlation demonstrated for the relations between visceral organ weights and lean body mass as well as empty body weight suggest that the weight of the liver, heart, kidney, spleen and pancreas have high predictive value in the calculation of these important factors. The use of organ weights as indices of lean-body mass and body weight provides additional indirect methods for the estimation of total body weight and the very important component of the body, the lean-body mass.

Summary

Data on 165 cattle have been studied statistically to determine the degree of association between the weights of the visceral organs, spleen, liver, heart, kidney and pancreas, and the weight of lean-body mass and empty-body weight. These organs were also evaluated as indicators in the estimation of empty-body weight and lean-body mass in cattle.

There is a very high positive correlation between the organ weights and the weights of the empty-body and the lean-body mass. All visceral organs studied were about equally reliable in their predictive value. For a population of cattle limited in age and weight, the liver appears to be a better indicator than the other organs. With a group of cattle varying more widely in age, weight and degree of fatness all organs are good indicators. The hypothesis proposed that liver is uniquely related to lean-body mass is invalidated since other organs are also highly correlated with this factor and because this relationship is influenced largely by total or empty-body weight.

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SELECTION FOR INCREASED WEIGHTS OF SIX-MONTH-OLD BEEF CALVES IN A BRAHMAN-ANGUS POPULATION [1]

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IMPROVEMENT of weaning weights in a Brahman-Angus population at Jeanerette, Louisiana, is being studied by the Bureau of Animal Industry cooperating with the Louisiana Agricultural Experiment Station. This paper must be considered in the nature of a preliminary or progress report since some phases of the study have only been in operation a short time and the number of animals available for study has been relatively small. The original crosses between the Brahman and Angus cattle at Jeanerette were started under the direction of the late W. H. Black, formerly in charge of the Beef Cattle Section of the U. S. Animal Husbandry Division. Results of early work with the crossbreds have been reported by Rhoad and Black (1943) in a circular on "Hybrid Beef Cattle for the Gulf Coast Region." Baker and Black (1950) published results of work with the crossbreds through 1949.

Materials and Methods

This report deals with comparable weights of 446 six-month-old calves obtained since 1945 and including the 1950 calf crop. From 10 to 23 sires and from 52 to 174 dams were involved in different phases of the study. The cows calved in January, February, and March. Weights were made on grass with no supplement except minerals.

The proportion of cows having different proportions of Brahman and Angus breeding varied somewhat in the herd by years from 1945 to 1950, figure 1. Baker and Black (1950) found that there were some differences in the six-months' weights of female calves from cows of these different proportions of Brahman and Angus breeding. However, many of the differences were not significant and for the purpose of this study all the groups were combined to give larger numbers.

Calf weights were markedly affected by years, sex of calf, and age of dam. Therefore the differences between the six-months' weights of

[1] This work is cooperative with the Louisiana Agricultural Experiment Station and has been part of the S-10 Project since 1949.

individual calves and the average six-months' weight for calves born in the same year, of the same sex, and from heifers or older cows have been used in this study rather than the actual weights. The actual average weights of the calves by years, and sex, and by age of dam from which the differences were derived are shown in figure 2. It will be noted that there was a wide variation in the average figures for the different years but that there was quite a consistent difference between

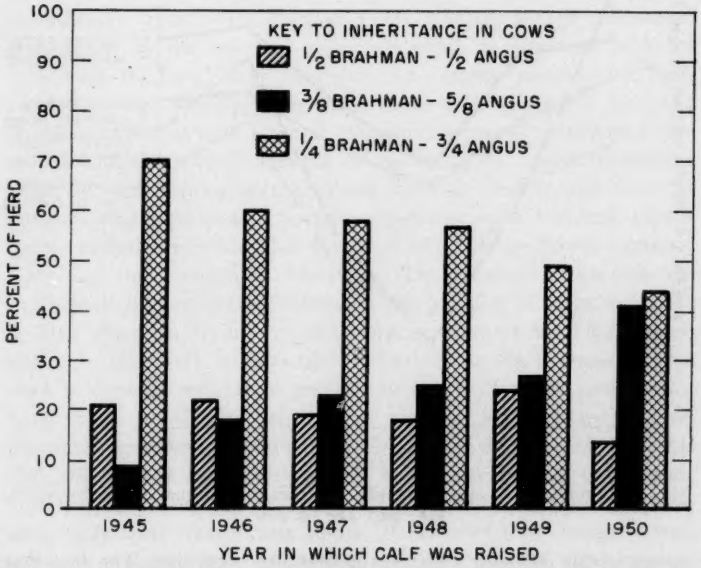

Figure 1. Proportions of cows of different degrees of Brahman and Angus inheritance in the herd at Jeanerette, La., by years.

male and female calves and between calves from heifers and older cows. Differences in six-months' weight associated with age of dam were studied previously by Baker and Black (unpublished material) for female calves. They found quite a consistent increase in weight of calf with increase in age of dam up to 6 years of age. However, the biggest difference was between calves from 3-year-old heifers and those from older cows. For the purpose of this study it was decided to separate the calves into only 2 groups, those from 3-year-old heifers and those from older cows, since the numbers were so small.

Results and Discussion

The regression coefficient for 6-month-weight of offspring on 6-month-weight of sire (all figures are on the basis of differences—see third paragraph under Materials and Methods) was slightly but not significantly negative (—.18). This is interpreted as indicating that there was on the average no apparent effect of the sire on the 6-month-

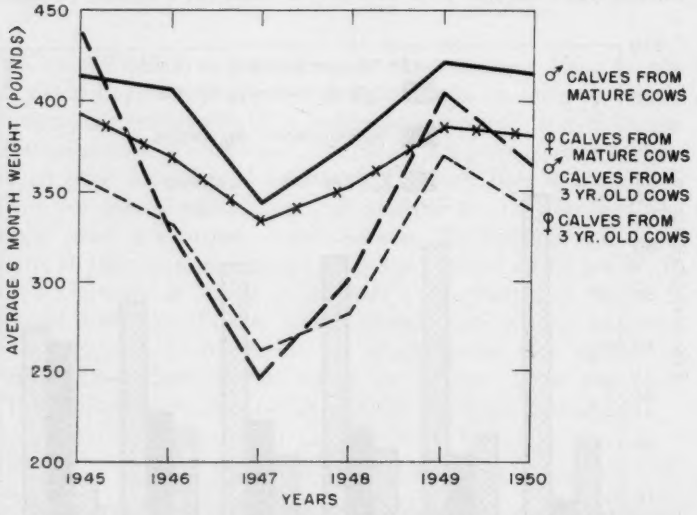

Figure 2. Average 6 month weight of Brahman-Angus calves by years, sex, and age of cow

weight of his offspring or a zero heritability coefficient. The fact that the sires used in this study were a highly select group so far as their own 6 months' weight was concerned may be a factor in reducing their apparent effect on the 6 months' weight of their offspring. When the average regression coefficient of 6-month-weight of offspring on 6-month-weight of dam (within sire of the offspring) was calculated, the regression was slightly positive (.02) and the estimated heritability was 5 percent. The average regression coefficient of 6-month-weight of offspring on 6-month weight of dam (within sire of the dam) was still more positive (.08) and the estimated heritability was 15 percent.

In addition an attempt was made to calculate the paternal half sib correlation but the analysis showed slightly more variance within the offspring of the same sires than between the offspring of different sires and so it was concluded again that the sires on the average were

having very little effect on the 6-months' weight of their offspring and that the heritability was zero.

The paternal half sib correlation was calculated for the daughters of the various sires for which data were available using the 6-months' weight of their calves as the criteria. From this an estimated heritability coefficient of 19 percent was obtained which agreed quite closely with the estimated 15 percent obtained from the dam daughter regression within the sire of the dam. This estimated heritability of 19 percent undoubtedly contains the fraction of the additive genetic inheritance transmitted by the maternal grandsires which is concerned with the mothering ability of their daughters, i.e., uterine environment, milk production and possibly other items involved in post-natal care, and it also contains at least some of the additive genetic inheritance concerned with the growth potential of the daughters' calves themselves. Since the sire-offspring regression was not even positive and since the dam-offspring regression is in part dependent upon maternal effects, the presumption is strong that the direct effect of the dam is relatively large and the transmitted effect low. The standard errors inherent in the heritability estimates themselves are probably so large that there is little point in trying to make precise separations. Furthermore, although there were no detectable differences on the average between sires in 6-month weights of progeny in this herd, other investigators have shown positive heritabilities of weaning weight based on sire-offspring regressions or half-sib correlations. It thus appears probable that the lack of sire effects in this study may be due to sampling variation or perhaps to the fact that the sires used were relatively uniform in their transmitting ability. If either of these things is true, our data probably tend to over-emphasize the relative importance of maternal effects.

The only other known reported study of the inheritance of maternal effects in beef cattle was among Hereford cattle at Miles City, Montana (Rpt. Chief, Bur. of An. Ind., 1949) where it was found that at weaning time the heritable effect of the maternal grandsire was about the same as the heritable effect of the sire of the calf insofar as weight was concerned.

To determine the relationship of the dam's previous calves to her subsequent calves, the average regression coefficient of the 1949 and 1950 6-month calf weight within sires on the average weight of their dam's previous calves at the time of mating was calculated and found to be .29. The average regression coefficient of the 1949 and 1950 6-month-old calf weight within sires on the weight of their dam's best

previous calf at time of mating was also .29. The correlation was .33 in both cases. It had been expected that the average of the previous calves would give a little higher correlation than the best calf with the 1949 and 1950 calf weight. One can assume that an exceptionally good calf has both a favorable heredity and a favorable environment, while an average calf may be retarded by a lack of either the one or the other. From this point of view there may be some merit in using the best calf a cow produces as a measure of her optimum ability. If such a measure is used it probably should be in conjunction with the average rather than alone. There were 111 comparisons or pairings that could be made between the 1949 or 1950 calves and the average of their dam's previous calves. There were the same number using their dam's best previous calf. However of the 111 there were 29 cases in which the values for the average and the best previous calf were the same. The mean for the dams' previous best calves was 18 pounds higher than the mean for the average of the dams' previous calves and the two regression lines were five pounds apart even though the slope of the lines was the same.

The estimates in this paper as well as those of other workers indicate a relatively low heritability of weaning weight within strains while observation indicates that wide differences occur between strains of cattle in this character. This suggests the possibility that, in addition to additive gene action included in conventional heritability estimates, dominance and epistasis may be of importance. If these were important, it might be difficult to obtain maximum weaning weights except in crossbred calves or in the calves produced by crossbred dams. Studies of the early generations of the population under study (Rhoad and Black, 1943; Rhoad, Phillips, and Dawson, 1945; and Baker and Black, 1950) indicated that the Brahman $\times$ Angus F_1 calves and the calves out of F_1 cows greatly exceeded calves of the Angus breed in six months weight. While no purebred Brahmans were raised in this study, the results suggested definite heterosis. Other work (Kidder and Chapman, 1952) indicates a heterotic effect on six months weight from crosses between the Brahman and British breeds. Since most theories as to the nature of heterosis assume non-additive gene action, it seems a reasonable assumption that non-additive types of inheritance were present in the early generations of the population under study and that they could be important in subsequent generations of *inter se* mating.

A few specific cases in the present study suggest that some sires were transmitting superior ability to grow and/or produce heavier calves

(figure 3). However, since differences among sire progenies were not significant, it cannot be inferred that they necessarily indicate non-additive types of inheritance.

The data in this paper indicate that heritability of weaning weight is low and that progress in improving it within a strain of cattle is likely to be slow. Maternal ability of the dam apparently exerts a more important influence on calf weight than the inheritance of the

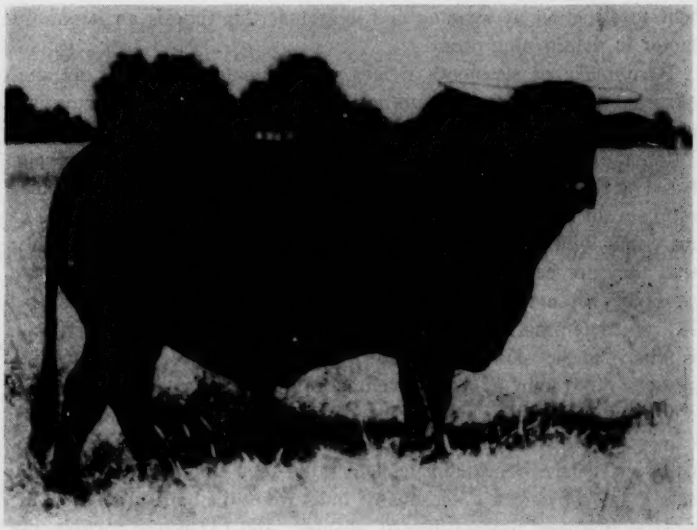

Figure 3. Half-bred Brahman-Angus sire 51Gl. This bull quite consistently produced offspring above average in weaning weight. His 23 daughters also produced calves that on the average were 8.5 pounds above the means for their groups.

calf itself. The heritability and repeatability of maternal ability are high enough to suggest that the following practices are desirable in selecting for weaning weight: (1) retain a high percentage of heifers for one or two calf crops and select those which demonstrate their ability to wean heavy calves for further use in the herd, (2) select sires from among the sons of cows which have repeatedly demonstrated their ability to wean heavy calves, and which are grandsons of bulls whose daughters have on the average produced heavy calves at weaning, and (3) where possible use sires whose daughters have proven to have good maternal abilities. It is recognized that the latter suggestion is

impractical under many conditions but if a bull is still living when his daughters are shown to have good records, he should be exploited.

Summary and Conclusions

This report deals with the six-month weights of 446 calves produced during the years 1945–1950 at the U. S. Iberia Livestock Experiment Farm, Jeanerette, Louisiana in a strain of cattle derived from a Brahman-Angus crossbred foundation. In this area many calves are sent to slaughter at weaning and weight at this time is an important factor in determining profit.

Heritability of six-month weight was estimated as zero from sire-offspring regressions and paternal half-sib correlations of immediate offspring, and from 5 to 15 percent based on dam-offspring regressions. Paternal half-sib correlations among daughters of the sires used and based on the weights of calves raised by the daughters gave a heritability estimate of 19 percent. These figures indicate the importance of maternal abilities in determining six-month calf weights and suggest that they are heritable traits. The data presented do not exclude the possibility of non-additive types of gene action being important and indicate that progress in selecting for six-month weight within a strain is likely to be slow. The following selection procedures were suggested: (1) retain a high percentage of heifers for one or two calf crops and select those which demonstrate their ability to wean heavy calves for further use in the herd; (2) select sires from among the sons of cows which have repeatedly demonstrated their ability to wean heavy calves and which are grandsons of bulls whose daughters have on the average produced heavy calves at weaning; and (3) where possible, use sires whose daughters have proven to have good maternal abilities.

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HERITABILITY OF MILK PRODUCTION IN MILKING SHORTHORN CATTLE

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MILK production is one of the two main objectives in raising Milking Shorthorn cattle. It is highly desirable that the inheritance of this important character be estimated since this would reveal some information on the way the character is transmitted from one generation to the next and the extent of improvement in this character to be expected in selective breeding. The findings would also give the basis for drawing or improving breeding and selection plans. The estimates of the heritability of milk production, butterfat production and peak-daily-milk production in Milking Shorthorn cattle are reported in this paper.

Material and Methods

The milk, butterfat and peak-daily-milk production records from 163 Milking Shorthorn cows raised and milked at the Agricultural Research Center, Beltsville, Maryland, were used in this study. They were all first lactation records obtained from 1934 through 1951, with two milkings a day. Milk production was recorded for each milking. Butterfat percentage was determined once a month and butterfat production for the month was calculated from this percentage. Lactation varied in length from 22 days to 305 days since the cows producing less than 10 pounds of milk per day were dropped from the test and the production records beyond 305 days were not counted. Peak-daily-milk production was the highest daily record during the whole lactation.

The cows were the offspring of 21 sires and 109 dams. Records available on both dam and daughter were for only 123 cows out of the 163 animals mentioned above and these were the offspring of 15 sires and 75 dams. These records on the 123 cows were secured from 1939 through 1951 and were used to calculate the daughter on dam regression.

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Analysis of variance and covariance methods described by Fisher (1946) and Snedecor (1948) were used to separate the total variances and covariances into different components due to different sources. The variances between sires within years and the variances within sires within years were used in the paternal half-sib correlation method, and the variances and covariances within sires within years were used in the intra-sire daughter-on-dam regression method as Lush (1949) suggested to estimate the heritability of milk, butterfat and peak-daily-milk production.

The constant for the average size of sire groups (k) used in the paternal half-sib correlation method was calculated with the formula $\frac{(\Sigma k)^2 - \Sigma k^2}{\Sigma k\,(n-1)}$, in which k = number of offspring in each sire-within-year group, n = number of sire-within-year groups, as used by Hetzer, Dickerson and Zeller (1944).

The standard errors for the heritability estimates obtained with the paternal half-sib correlation method and the intra-sire regression of daughter on dam method were estimated with the formulas given by Hazel and Terrill (1945). The standard errors for the heritability estimates obtained by the paternal half-sib correlation method are not unbiased estimates due to the sires being repeated in more than one year and the actual standard errors would be somewhat higher than the estimates given.

Results and Discussion

The material used in this study was of one breed and one herd, and the animals were treated as equally as possible. Most of the sires used were born and raised in the same herd. Close inbreeding was intentionally avoided so the inbreeding coefficient of the individual animal would not often be higher than 1.6 percent which should not materially affect the results of the heritability estimates. The actual lactation length for the individual cows varied from 22 to 305 days and was regarded as one of the closely related characters to the milk production. If the milk production were corrected to a certain length of lactation period, calculating errors might be involved and part of the production variance due to the genetic influence from the last generation would be lost. Therefore, in this study, no correction was made in the production for the difference in lactation length.

Differences in the mean variances due to years for the three milk production characters were all highly significant in the 163 cows (P less

than 0.01) while in the 123 cow group they were moderately significant (P less than 0.05). See tables 1 and 2. The difference in the significance for the two groups may have been partly due to the reduction in the number of years, from 18 in the larger group to 13 in the smaller. It is also probable that it was partly due to a decrease in the variability in the later years of the experiment as was indicated by the decrease in the size of the mean squares between years (table 2 compared to table 1). However, in the case of the production for the dams of the 123 cows (table 2) the mean variances due to years were not significant. This is probably because the yearly differences are confounded with differences between dams since the dams were sorted on the basis of the years in which the daughters made their records.

TABLE 1. ANALYSES OF VARIANCE FOR MILK, BUTTERFAT AND PEAK-DAILY-MILK PRODUCTION OF 163 MILKING SHORTHORN COWS

		Mean Squares			
Source	d.f.	Milk	Butterfat	Peak-Daily-Milk	k-Value
Total	162	3,944,197	6,058	56.2	
Year	17	9,154,402**	12,696**	124.1**	
Sire within year	31	4,331,253	6,581	53.6	3.31
Error [1]	114	3,456,722	4,926	46.7	

** Highly significant at 1 percent level.
[1] Within sire and within year.

Differences in the mean variances due to sires within years for the three milk production characters were not significant in the 163 cow group, or the 123 cow group, tables 1 and 2.

Heritability coefficients for milk, butterfat and peak-daily-milk production ranged from 14.2 to 36.8 percent when estimated from the additive portion of the heritability using the paternal half-sib correlation method (table 3). When they were estimated with the intra-sire regression of daughter on dam method they ranged from 71.1 to 83.9 percent.

The estimated heritabilities from the paternal half-sib correlation for the 163 cows were somewhat higher than those for the 123 cows. These differences may be due to the reduction in the number of sires and the increase of the uniformity in the sire potential milk production in the later years. Also the 40 cows on which there were no records for the dams did not represent a random sample from the population. Most of them were from the earlier years of the experiment and thus subject to a different selection pressure than the population as a whole. Sampling

TABLE 2. ANALYSES OF VARIANCE AND COVARIANCE FOR MILK, BUTTERFAT AND PEAK-DAILY-MILK PRODUCTION OF 123 MILKING SHORTHORN COWS AND THEIR DAMS

Production	Sources	d.f.	Mean Squares		Mean Cross-Products
			Daughters	Dams	
Milk	Total	122	4,201,302	2,621,392	1,406,976
	Year	12	8,068,192*	3,366,101	3,333,826
	Sire within year	24	4,119,712	2,399,809	999,395
	Error [1]	86	3,672,877	3,518,693	1,251,857
Butterfat	Total	122	6,031	3,880	1,897
	Year	12	10,324*	5,471	4,483
	Sire within year	24	6,462	3,257	1,642
	Error [1]	86	5,312	3,832	1,608
Peak-daily-milk	Total	122	57.3	40.8	16.9
	Year	12	106.5*	65.5	65.3
	Sire within year	24	57.0	33.7	1.3
	Error [1]	86	50.5	39.4	14.5

* Significant at 5 percent level.
[1] Within sire and within year.

errors due to the limited amount of data may also be one of the sources of the differences.

There were large differences between the heritability estimates obtained by the paternal half-sib correlation method and the intra-sire regression of daughter on dam method for each of the three characters (table 3). The higher heritability estimates obtained from the daughter on dam regression may have been partly due to epistatic effects, to some selectional influences, and to sampling errors which may occur due to the

TABLE 3. HERITABILITY COEFFICIENTS OF MILK, BUTTERFAT AND PEAK-DAILY-MILK PRODUCTION IN MILKING SHORTHORN CATTLE

Method	No. cows	Milk		Butterfat		Peak-daily-milk	
		H.	s.e.	H.	s.e.	H.	s.e.
Paternal half-sib correlation	163	28.4	31.5	36.8	32.5	16.9	30.6
Paternal half-sib correlation	123	14.2	35.2	24.5	36.0	14.8	35.1
Intra-sire regression daughter on dam	123	71.1	29.8	83.9	23.7	73.7	23.0

H. = Heritability.
s.e. = Standard error.

limited number of data. There is also the possibility that they may have been influenced by certain maternal effects. Epistatic effects are difficult to demonstrate but some of the selection influences can be pointed out.

The use of sires in the herd which came from dams with high milk production records and which had high producing daughters should, if selection is effective, tend to make the sires more alike in their potential milk production and thus cause a reduction in the apparent heritability of the traits. The number of sires used in the herd is only about one-fifth of the number of dams. Therefore, a more intensive selection of sires than of dams has been possible. On the other hand, Milking Shorthorns are dual-purpose cattle and emphasis in selecting bulls might, therefore, to some extent, be put on characters favorable to beef production and retain many of the genes for milk production in a heterozygous condition. This might reduce the potential milk production of the sires. The portion of the variance caused by the sires in either case would be small and the estimates of heritability from the paternal half-sib correlation would be reduced.

Nearly all the heifer calves born in the herd were raised for milk production and what culling there was is believed to have been largely at random so far as milk production was concerned. Of course, more of the heifers came from good dams than from poor ones since the poorer dams were culled from the herd before they could produce many daughters. The fact that the average production in the herd did not increase during the period of study indicates that either the actual heritability of the traits was not high enough to make such selection effective or that there was an opposing selection, i.e. for beef characters, which offset the selection for milk production. There is some evidence that an improvement was made in four generations in the total amount of lean and in the lean-bone ratio in the Milking Shorthorn steers slaughtered at a final weight of 900 lb.

Intra-uterine effects might also influence milk production of the daughters. It seems unlikely that this would increase the correlation between the production of the daughter and the production of the dam, as compared to that obtained from paternal half sibs, unless both daughter and dam had been subjected to similar maternal influences. Since there is some evidence that at least part of the maternal influence is of an hereditary nature, this is possible, Dawson, Vernon, Baker and Warwick (1954).

Maternal effects in which some elements in the cytoplasm of the

cattle egg cells are directly transmitted from the dams to the offspring may have modified or increased the activities of the milk production genes and brought up the regressional relationship between daughters and dams for these characters. Maternal effects of this kind have been demonstrated to exist in a number of species (Sonneborn, 1951; Caspari, 1948) and it is an interesting speculation that some may exist in cattle.

Since there are a number of factors which would tend to increase the heritability of the milk production characters when estimated by the intra-sire daughter-on-dam regression method and a number of factors which would tend to decrease the heritability of the milk production characters when estimated by the paternal half-sib correlation method, the authors are inclined to believe that the true heritability for the additive portion of the inheritance lies between the values found by the two methods.

Summary

The first lactation milk production records from 163 Milking Shorthorn cows raised and milked at the Agricultural Research Center, Beltsville, Maryland, from 1934 through 1951, were analyzed to determine the heritability estimates of milk, butterfat and peak-daily-milk production for this herd. The paternal half-sib correlation method was used with the 163 cows. Both the paternal half-sib correlation method and the intra-sire daughter on dam regression method were used with 123 of the 163 cows on which records were available on both the daughters and dams.

The heritability coefficients for milk, butterfat, and peak-daily-milk production were 28.4, 36.8 and 16.9 percent for the 163 cows derived from the paternal half-sib correlation method. They were 14.2, 24.5 and 14.8 percent from the paternal half-sib correlation method and 71.1, 83.9 and 73.7 percent from the intra-sire daughter on dam regression method for the 123 cows.

Possible causes for the differences found between the estimates obtained from the paternal half-sib correlations and those from the intra-sire daughter-on-dam regressions were discussed. The authors conclude on the basis of the results and of this discussion that the true heritability for the additive portion of the inheritance for the three milk production characters in this herd lies between the values found by the two methods.

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THE EFFECT OF CARBOHYDRATES AND SULFUR ON B-VITAMINS SYNTHESIS, CELLULOSE DIGESTION, AND UREA UTILIZATION BY RUMEN MICROORGANISMS *IN VITRO*[1, 2]

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IT is well known that members of the vitamin B-complex are synthesized in the rumen of the bovine, but our knowledge of the factors which affect these syntheses are rather limited. In recent studies, Burroughs *et al.* (1950, 1950a, 1951) have shown that rumen microorganisms can be used to study cellulose digestion and urea utilization in an artificial rumen. It was thought that this type of apparatus would lend itself admirably to a study of various nutrients as they may affect the synthesis of these vitamins and also the effect on cellulose digestion and urea utilization.

Loosli *et al.* (1949) and Thomas *et al.* (1951) have reported that elemental sulfur and Na_2SO_4 in a sheep ration increased urea nitrogen retention and the synthesis of sulfur containing amino acids in the rumen. Block *et al.* (1950, 1951) have found that radioactive sulfur supplied as Na_2SO_4, in rations fed to cows and goats, appeared in the milk. Garrigus *et al.* (1950) fed elemental sulfur to sheep maintained on a methionine deficient ration and observed that wool production was increased, but growth was not stimulated. Starks *et al.* (1953) reported that elemental sulfur was beneficial in the utilization of nitrogen from urea when fed to sheep on a low sulfur ration, while Hale and Garrigus (1953) found that sheep could synthesize cystine when fed radioactive sulfur, either as elemental or sulfate sulfur. They also found that sulfate sulfur was better utilized in the synthesis of cystine than elemental sulfur. Cline (1952) made *in vitro* studies on the effect of sulfur compounds and concluded that Na_2SO_4, methionine, and Na_2SO_4 plus cystine stimulated cellulose digestion and protein synthesis. Keener *et al.* (1953) found that radioactive (S^{35}) sulfur was incorporated into the proteins of the blood and milk when $S^{35}O_2$

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preserved silage was fed to lactating cows. Hunt *et al.* (1952) made a preliminary *in vitro* study of the effect of starch and noted an increased synthesis of certain members of the vitamin B-complex. So far no work has been reported on the effect of sulfur-containing compounds on the synthesis of the B-complex vitamins by rumen microorganisms. This study was an endeavor to investigate these relationships.

Experimental Procedure

The experimental procedures were the same as those used by Burroughs and associates (1951, 1951a), and Bentley and associates (1954). The inoculum was obtained from two fistulated steers maintained on either good alfalfa hay or poor quality timothy hay. The liquid portion of the rumen ingesta was filtered through four thicknesses of cheesecloth and then added to each flask, 25 percent by volume. At the end of each experiment the flask contents were made to volume (1 liter) and then the desired samples were taken for vitamin assay, cellulose, and urea (NH_3) determinations. The vitamin assay results were all based upon the total amount found in the digestion flasks (artificial rumen) at the end of the period minus the amount added by means of the inoculum. The cellulose and urea were reported as percent digested and mgs. NH_3 utilized, respectively.

Riboflavin

The samples from each flask were prepared by adding 25 ml. 0.2 N Hcl to each of 25 ml. sample (conc. now 0.1N) and then autoclaved for 15 minutes at 15 pounds pressure. To the cooled sample was added 3.3 ml. 2.5 molar sodium acetate and made to volume. The samples were then filtered and the filtrates adjusted to pH 6.6 to 6.8. Suitable aliquots were taken for assay. The microbiological assay method of Snell and Strong (1939) was followed using *Lactobacillus caseii.*

Pantothenic Acid

These samples (25 ml.) were diluted to 75 ml. (pH 6.5–6.8) with distilled water and autoclaved for 30 minutes at 15 pounds pressure, then cooled and made to volume, filtered through filter paper, and suitable aliquots were assayed according to the method previously used in this laboratory (Hunt *et al.* 1947), using *Lactobacillus arabimosus* 17–5 as the test organism. This procedure measured the free pantothenic acid. The total pantothenic acid was determined by the same method

after enzymatic hydrolysis for 24 hours at 37° C, using 20 mgs. each of takadiastase and papain per sample, Childelin *et al.* (1942). Four to five times more pantothenic acid was released by this enzymatic procedure, but there is no assurance that this represents the total amount, in the light of recent studies on the enzymatic hydrolysis of coenzyme A (Novelli *et al.*, 1949).

Niacin

The 25 ml. samples were diluted with 25 ml. of 2 N NaOH (conc. now 1 N) and autoclaved 30 minutes at 15 pounds pressure. The samples were cooled, neutralized to pH 6.6 to 6.8, made to volume and then allowed to stand one hour or more to allow the precipitate to settle. Suitable aliquots of the supernatant were taken and assayed according to the method previously used in this laboratory (Hunt *et al.*, 1947).

Vitamin B_{12}

Twenty-five ml. aliquots were taken and analyzed for B_{12} by the method of Skeggs *et al.* (1950), modified by using sodium ascorbate as the reducing agent to protect B_{12} during sterilization. *Lactobacillus leichmanni* ATCC-7830 was the test organism.

Results and Discussion

Effect of Starch

The data from a series of experiments which are presented in table 1 were subjected to an analysis of variance with results as shown in table 2. The analyses show that starch had a highly significant effect on the synthesis of riboflavin, the digestion of cellulose, and the utilization of urea. The data in table 1 show that starch depresses significantly the digestion of cellulose. The fact that starch depressed cellulose digestion confirms a previous report from this Station (Arias *et al.*, 1951) and bears out the fact, as stated by them, that the microorganisms will obtain their energy requirement from the more readily available source instead of breaking down the cellulose. The starch did not significantly effect the synthesis of vitamin B_{12}.

Effect of Time

The fermentation time had a highly significant effect on the synthesis of riboflavin (48 hour period lower than 24 hours), the digestion of

TABLE 1. THE RELATIONSHIP BETWEEN THE SYNTHESIS OF RIBOFLAVIN AND VITAMIN B_{12}, CELLULOSE DIGESTION, AND UREA UTILIZATION AS AFFECTED BY STARCH

Products of Digestion by Inoculum Alfalfa Hay Fed Steer	No Starch			Starch[1]		
	24 Hours	48 Hours	96 Hours	24 Hours	48 Hours	96 Hours
Riboflavin synthesized, μg (a)	104 (2)	39 (2)	145 (11)	253 (4)	98 (3)	382 (10)
Vitamin B_{12} synthesized, μg (b)	5.6 (3)	8.0 (3)	1.9 (5)	14.1 (3)	12.7 (3)	3.0 (5)
Cellulose digested, %	36.9 (10)	65.1 (10)	66.3 (14)	28.7 (10)	57.5 (9)	57.0 (16)
Ammonia utilized, mgs.	329 (11)	424 (14)	422 (15)	491 (11)	588 (14)	619 (14)
Inoculum—poor hay fed steer						
Riboflavin synthesized, μg (a)	115 (5)	35 (3)	168 (8)	233 (6)	105 (4)	598 (6)
Vitamin B_{12} synthesized, μg (b)	4.6 (2)	1.4 (3)	0.8 (5)	5.7 (2)	5.1 (3)	1.14 (5)
Cellulose digested, %	19.0 (8)	48 (7)	45.0 (15)	9.6 (7)	11.8 (6)	36.4 (15)
Ammonia utilized, mgs.	288 (11)	424 (11)	367 (16)	388 (13)	468 (11)	574 (16)

(a) Av. riboflavin content, (alfalfa hay) inoculum added, 63.00 μg.
(a) Av. riboflavin content, (poor hay) inoculum added, 41.00 μg.
(b) Av. vitamin B_{12} content, (alfalfa hay) inoculum added, 1.38 μg.
(b) Av. vitamin B_{12} content, (poor hay) inoculum added, 0.58 μg.
[1] 9.0 gm. starch per flask.
The figures in parenthesis indicate the number of replicas run to obtain these data.

TABLE 2. ANALYSIS OF VARIANCE FOR RIBOFLAVIN, VITAMIN B_{12}, CELLULOSE DIGESTED, AND MGS. NH_3 UTILIZED IN *"IN VITRO"* DIGESTION

Source of Variance	Riboflavin			Vitamin B_{12}			Cellulose Digested			Mgs. NH_3 Utilized		
	D.F.	Mean Square	F	D.F.	Mean Square	F	D.F.	Mean Square	F	D.F.	Mean Square	F
Starch	1	585,582	39.8‡	1	86.3	0.49	1	3,645	10.9‡	1	917,006	12.1‡
Time	2	229,374	15.6‡	2	170.3	0.97	2	8,272	24.8‡	2	218,840	28.8‡
Inoculum	1	22,058	1.5	1	181.7	1.04	1	14,030	42.0‡	1	49,456	6.5‡
Group	11	141,242	9.6	11	66.0	0.38	11	3,544	10.6	11	146,648	19.3
Interaction	7	69,611	4.7	7	16.8	0.09	7	485	1.45	7	29,855	3.9
Error	41	14,704		19	174.8		104	334		136	7,577	
Total	63			41			126			158		

‡ Highly significant, probability less than 0.01.

cellulose and the utilization of urea. Time did not appear to be a factor in vitamin B_{12} synthesis.

Effect of Inoculum

The results show (table 1) that more cellulose was digested and more urea was utilized when the inoculum was obtained from the

TABLE 3. EFFECT OF SOURCE OF INOCULUM, STARCH, AND SULFUR COMPOUNDS ON THE SYNTHESIS OF SOME MEMBERS OF THE VITAMIN B-COMPLEX

Distinguishing Features of the Artificial Rumen Substrate—96-Hour Digestion	Vitamins Synthesized per Flask, Micrograms [2]			
	Riboflavin	Niacin	Pantothenic Acid [1]	B_{12}
Inoculum (alfalfa hay fed steer)				
1. 0.0 gm. starch	131	502	—11.0	1.80
2. 9.0 gm. starch	297	1658	43.0	3.74
3. 0.0 gm. starch + 100 mgs. methionine	171	509	— 7.0	2.05
4. 9.0 gm. starch + 100 mgs. methionine	389	1326	1.0	3.84
5. 0.0 gm. starch + 150 mgs. Na_2SO_4	160	542	—11.0	5.47
6. 9.0 gm. starch + 150 mgs. Na_2SO_4	599	4070	68.0	13.46
7. 9.0 gm. starch + 50 mgs. glutathione	551	1649	12.0	1.65
Inoculum (poor hay fed steer)				
1. 0.0 gm. starch	185	684	— 1.0	0.37
2. 9.0 gm. starch	689	4709	1090.0	0.41
3. 9.0 gm. starch + 150 mgs. Na_2SO_4	523	3377	618.0	0.009
4. 9.0 gm. starch + 50 mgs. glutathione	353	1721	208.0	1.02
5. 9.0 gm. starch + 1 mg. aureomycin	—2	11	—19.0	0.44
Inoculum added (alfalfa hay)	43	233	39	0.73
Inoculum added (poor hay)	31	187	26	0.67

[1] Free pantothenic acid.
[2] Total found minus vitamins added via inoculum.

alfalfa hay fed steer than from the poor hay fed steer. These results are highly significant (table 2). It appears that the alfalfa hay ration supplied nutrients for the microorganisms which stimulated their activity or supported a greater population than the nutrients from the poor hay. This effect may be related to the riboflavin, vitamin B_{12}, sulfur content or unknown factor(s) in the alfalfa hay.

Effect of Sulfur

The results of an experiment summarized in table 3 show that sulfur as Na_2SO_4 and sulfur as methionine, together with starch, increased the

amount of riboflavin, niacin, and vitamin B_{12} synthesized over and above those without sulfur. There was an increased amount of free pantothenic acid synthesized when starch was added, irrespective of the source of the inocula, but the effect of sulfur compounds was not conclusive. Gluthathione as a source of sulfur appeared to have no stimulatory effect on the synthesis of the B-vitamins studied. The results also show that more vitamin B_{12} was synthesized in the flasks using the inoculum from the alfalfa hay fed steer than from the poor hay fed steer. One mg. aureomycin almost completely inhibited the growth of the microorganisms which synthesized riboflavin, niacin, and pantothenic acid, but appeared to have no effect on the organisms responsible for the synthesis of vitamin B_{12}.

Until this time in the study the source of the inoculum for each flask was the liquid portion of the rumen content of the steer. In the following experiments the filtered liquid portion of the rumen content of the steer fed alfalfa hay was run through a Sharples supercentrifuge and in this way separated into two fractions, viz. the liquid and cells. The cells contained the greater portion of the mircoorganisms. These microorganisms were suspended in a phosphate buffer (pH 7.0) and this suspension together with the liquid fraction constituted the inoculum. The object of this procedure was to find, if possible, the essential fraction of the inoculum which was involved in the results obtained. The digestion results as obtained from an experiment with this type of inoculum and sulfur from four different sources are shown in table 4.

It is very evident from these data that sulfur as found in methionine and Na_2SO_4 stimulated the synthesis of riboflavin and vitamin B_{12}, the digestion of cellulose and the utilization of urea to a greater extent than cystine or elemental sulfur. These findings seem to parallel previously reported results of feeding experiments with ruminants as reviewed in this paper. It is very evident from the above data that the microorganisms involved have a definite sulfur requirement. The observation that elemental sulfur stimulated the synthesis of riboflavin only to a limited extent and did not stimulate the digestion of cellulose, the utilization of urea, nor the apparent inhibition of pantothenic acid synthesis leads to the conclusion that elemental sulfur can be used as such or is converted to a form that can be used by microorganisms synthesizing vitamin B_{12} and to a lesser extent riboflavin, but cannot be used by microorganisms involved in the digestion of cellulose or the

utilization of urea. This could either be a requirement phenomenon or a solubility phenomenon.

Another striking fact is that all of the sulfur compounds depressed the synthesis of pantothenic acid. In most instances pantothenic acid synthesis proceeded at about the same rate when elemental sulfur was added as in the control flask but synthesis was apparently inhibited

TABLE 4. EFFECT OF SOURCE OF SULFUR ON THE SYNTHESIS OF RIBOFLAVIN, PANTOTHENIC ACID, VITAMIN B_{12}, AND CELLULOSE —DIGESTION AND UREA UTILIZATION

Distinguishing Feature of Artificial Rumen Substrate [1]	Synthesis/Flask, μg [3]			Cellulose, Digested, %	NH_3 Utilized, mgs.
	Riboflavin	Pantothenic Acid [2]	B_{12}		
Liquid + cells, 30 hr. digestion					
1. 0.0 gm. sulfur	23.3	128.0	0.7	13	177
2. 50.0 mgs. S. as methionine	231.3	24.0	2.5	60	237
3. 100.0 mgs. S. as methionine	259.3	76.0	2.6	60	249
4. 50.0 mgs. S. as cystine	131.3	72.0	1.9	28	145
5. 100.0 mgs. S. as cystine	71.3	44.0	1.9	17	105
6. 34.0 mgs. S. as Na_2SO_4	163.3	52.0	1.9	56	259
7. 50.0 mgs. S. as Na_2SO_4	215.3	60.0	2.6	56	257
8. 100.0 mgs. S. as Na_2SO_4	251.3	40.0	2.7	59	249
9. 34.0 mgs. S. as Na_2SO_4 + no starch	131.3	44.0	1.0	64	165
10. 50.0 mgs. elemental sulfur	75.3	116.0	0.2	12	41
11. 100.0 mgs. elemental sulfur	95.3	140.0	3.0	18	69
12. Cells only—no sulfur	35.3	12.0	−2.2	7.5	21
Vitamin content liquid added	40.0	8.0	1.7		
Vitamin content cells added	68.7	24.0	4.5		

[1] 4.5 gms. starch per flask unless otherwise stated.
[2] Total pantothenic acid.
[3] Total vitamins found minus vitamins added via inoculum.

by sulfur compounds which stimulated the other reactions. This supports the assumption that pantothenic acid is synthesized by different microorganisms than those responsible for riboflavin synthesis, cellulose digestion, and urea utilization. Furthermore, it would appear that the pantothenic acid synthesizing microorganisms have a lower sulfur requirement than those responsible for the other reactions studied or were in a relatively weaker competitive position when other microorganisms were stimulated by the addition of sulfur compounds to the substrate. The pantothenic acid synthesized may be utilized by the

microorganisms producing riboflavin, digesting cellulose and/or utilizing urea to form nitrogen compounds.

The fact that the cells fraction of the inoculum stimulated very poorly the synthesis of the vitamins, the digestion of cellulose and utilization of urea would indicate that the liquid fraction contains a substance(s) which are essential for good digestion *in vitro*.

The above experiment was repeated twice, with certain modifications, with the results of one as shown in table 5. Here again the results show that sulfur as found in methionine and Na_2SO_4, with a constant

TABLE 5. EFFECT OF THE SOURCE OF SULFUR ON THE SYNTHESIS OF RIBOFLAVIN AND PANTOTHENIC ACID, CELLULOSE DIGESTION, AND UREA UTILIZATION

Distinguishing Feature of Artificial Rumen Substrate [1]	Synthesized/Flask, μg [3]		Cellulose Digested, %	NH_3 Utilized, mgs.
	Riboflavin	Pantothenic Acid [2]		
Liquid + cells, 30 hr. digestion				
1. 0.0 mgs. sulfur	34.7	58.0	9.5	27.0
2. 50.0 mgs. sulfur as Na_2SO_4	170.7	42.0	50.0	239.0
3. 50.0 mgs. sulfur as methionine	267.7	62.0	52.0	243.0
4. 50.0 mgs. sulfur as cystine	126.7	38.0	20.0	127.0
5. 50.0 mgs. elemental sulfur	2.7	110.0	14.0	51.0
6. 50.0 mgs. sulfur as ethionine	−45.7	26.0	7.8	0.0
7. 34.0 mgs. sulfur as Na_2SO_4 + cells only	207.5	33.2	20.7	167.0
Vitamin content liquid added	48.8	15.2		
Vitamin content cells added	40.5	22.8		

[1] 4.5 gms. starch per flask.
[2] Total pantothenic acid.
[3] Total vitamins found minus vitamins added via inoculum.

source of energy (4.5 gm. starch/flask) gave the best response in the synthesis of riboflavin, the percent of cellulose digested and the urea utilized, of any sulfur compound used. With the exception of methionine, the sulfur compounds depressed the synthesis of pantothenic acid.

In the third experiment, the amount of pantothenic acid synthesized per flask was as follows: No sulfur, 70μ; elemental sulfur, 102μ; sulfur at Na_2SO_4, 14μ; sulfur as methionine, 6μ; and sulfur as cystine, 24μ. These results support the previous ones in which it was shown that the sulfur compounds depressed the synthesis of pantothenic acid.

The results also show (table 5) that ethionine alone acted as a antimetabolite and reduced the activity of the rumen microorganisms.

Summary

Starch as a source of energy (9.0 gm. starch/flask) as used in this type of artificial rumen stimulated the synthesis of riboflavin, niacin, pantothenic acid, and vitamin B_{12} and also increased the utilization of urea, but depressed cellulose digestion. A smaller amount of starch (4.5 gm./flask) was not sufficient for the best synthesis of the above vitamins and urea utilization, but supported better cellulose digestion.

The inoculum from the steer getting alfalfa hay was more effective in the synthesis of vitamin B_{12}, cellulose digestion, and urea utilization than the inoculum from the poor hay fed steer.

The results from the 24 and 30 hour digestion studies were found to be as significant in determining the results of digestion as the 48 and 96 hour.

Inorganic sulfur as Na_2SO_4 and sulfur in methionine stimulated the activity of the rumen microorganisms with the result that more riboflavin and vitamin B_{12} were synthesized, more cellulose was digested, and more urea utilized than when the source of sulfur was cystine or elemental sulfur. The data obtained indicate that pantothenic acid was synthesized by different microorganisms than those responsible for the other reactions studied in this experiment. This organism apparently has a low sulfur requirement.

Ethionine as the source of sulfur depressed the synthesis of all of the products studied.

The microorganisms which synthesize riboflavin, niacin, and vitamin B_{12}, digest cellulose, and utilize urea for protein synthesis have a sulfur requirement.

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STUDIES ON FACTORS NEEDED BY RUMEN MICROORGANISMS FOR CELLULOSE DIGESTION *IN VITRO* [1, 2]

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NUMEROUS experiments have shown that ration composition can influence microbial activity in the rumen of cattle and sheep. Cellulose digestion was depressed by feeding starch to steers (Burroughs *et al.*, 1949); whereas rations rich in "readily available carbohydrates" improved the synthesis of B-vitamins (McElroy and Goss, 1939; Hunt *et al.*, 1941) and the utilization of urea (Mills *et al.*, 1942). Also, the type of microorganisms present in the rumen of animals fed a ration high in roughage feeds differ noticeably from those found in the rumen if the ration is rich in grains (Pounden and Hibbs, 1948; Masson, 1950; Gall *et al.*, 1953). Further confirmation of these relationships has been demonstrated with rumen microorganisms *in vitro* (Pearson and Smith, 1943; Arias *et al.*, 1951).

Certain unidentified factors have been shown to stimulate the activity of rumen microorganisms *in vitro* (Burroughs *et al.*, 1950; 1951; Hungate, 1950; Huhtanen *et al.*, 1952; McNeill *et al.*, 1954). Doetsch, *et al.* (1952) found that the respiration rate of washed cell suspensions was greater if the substrate contained rumen juice. Recently, Ruf *et al.* (1953) reported that an extract of cow manure as well as several feedstuffs stimulated the rate of cellulose digestion if they were added to a purified medium used for the *in vitro* culture of rumen microorganisms. Bentley *et al.* (1953) found that a combination of B-vitamins, purines, uracil, and alfalfa ash increased cellulose digestion *in vitro*. Likewise, Hall *et al.* (1953) found that biotin and vitamin B_{12} increased cellulose digestion in their *in vitro* experiments. That an isolated culture of rumen microorganisms has complex vitamin and other growth factor requirements was indicated in a report by Wasserman *et al.* (1953).

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The results to be presented in this paper demonstrate the importance of certain B-vitamins, purines, and uracil for rumen microorganism activity and provide additional evidence for the existence of undetermined nutritional factors which are required by rumen microorganisms grown *in vitro*. Since these factors have a marked effect on cellulose digestion, it has been convenient to refer to them as "cellulolytic factors" with the full realization that there may be additional specific effects of these materials on rumen microorganism activity *in vitro*, e.g. ammonia utilization.

Experimental

Procedure

In vitro fermentation experiments with rumen microorganisms were carried out in liter low actinic Erlenmyer flasks which were arranged in a thermostatically controlled water bath (38–39° C.) to permit individual gassing of the flasks with carbon dioxide. The flask stoppers were fitted with two tubes and one, which was used to admit the carbon dioxide, extended to the bottom of the flask. The other tube, which extended only through the stopper, served as a vent. The length of the fermentation was from 24 to 48 hours and no additions, other than sodium carbonate, were made to the medium during the experiments. At the end of the experiments, the flask contents were made to volume, usually 1 liter, with water and thoroughly mixed to suspend the insoluble particles. Fifty ml. of the suspension was taken for cellulose determination using the method of Crampton and Maynard (1938). The amount of cellulose digested was used as the criterion for activity of the rumen flora as grown under the conditions of these experiments.

Basal Medium

The composition of the basal medium used in these investigations is described in table 1. The minerals with the exception of sodium carbonate and ferric chloride were prepared as a single mixture to facilitate the addition of the ingredients. The pH of the final medium was adjusted to 6.6–6.8 using a saturated sodium carbonate solution before the start of the experiment and at intervals throughout the fermentation. It was found that there was little change in pH after 6 to 10 hours with the basal medium. However, if starch or sugars were added to the medium, pH changes persisted for a longer period. This medium was essentially the same as previously described by Arias *et al.* (1951),

the modifications being in the amount of phosphate, sulfate, and iron added.

Inoculum

Each flask was inoculated with 75 ml. of a suspension which contained the microorganisms and some debris from 350 ml. of strained rumen juice obtained from a fistulated steer maintained on a ration composed only of good quality alfalfa hay. The strained rumen juice was centrifuged in a Sharples supercentrifuge (electrically driven) and the sediment was collected on a celluloid film which was fitted inside the cylinder. A pH 7.0 phosphate buffer composed of 1.059 gm. Na_2HPO_4, 0.436 gm. KH_2PO_4, and 0.05 gm. cysteine per liter was used to suspend

TABLE 1. COMPOSITION OF BASAL MEDIUM [1]

Constituent	gm./L	Constituent	gm./L
Cellulose [2]	9.00	$MgCO_3$	0.04
Urea	1.68	$CaCl_2$	0.04
Glucose	1.00	NaCL	0.43
Na_2HPO_4	1.13	Na_2SO_4	0.15
NaH_2PO_4	1.09	$FeCl_3$. 6 H_2O	0.044
KCL	0.43		
Inoculum—Sediment suspension, 75 ml. per liter			

[1] A solution which contained all the minerals except the $FeCl_3$ was prepared in such a concentration that 140 ml. per flask supplied the proper amount of minerals.
[2] Solka-Floc 40 A—Brown Company, Berlin, New Hampshire.

the cells. In this paper the term "sediment" will refer to the above described inoculum and "supernatant" will refer to the centrifugate from the Sharples which by itself was found to be ineffective as an inoculum in the flasks. On the average, 75 ml. of the inoculum contained 0.0027 gm. of ammonia nitrogen and 0.038 gm. of cellulose.

Results

Cellulolytic Factors

The rate of cellulose digestion was slow when the basal medium described in table 1 was used—the substrate inoculated with the sediment. However, the addition of 100 ml. of autoclaved rumen juice or supernatant to the medium gave the same rate of cellulose digestion as obtained when whole un-centrifuged rumen juice was used to inoculate the flasks. This observation led to an investigation of other

natural products, including feeds commonly fed to cattle, as possible sources of the cellulolytic factors. The results of this study are given in table 2. The increase in percent cellulose digestion obtained by the

TABLE 2. THE EFFECT OF VARIOUS NATURAL MATERIALS ON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS *IN VITRO*

Additions to Basal Medium	Amount Added, gm.	Relative Activity
None (Sediment)	..	0
Rumen juice (Supernatant)	100	100
Hot H_2O extract alfalfa leaf meal [1, 2]	1	40
Hot H_2O extract alfalfa leaf meal	2	40
Hot H_2O extract alfalfa leaf meal	5	75
Hot H_2O extract alfalfa leaf meal	10	101
Hot H_2O extract good timothy hay	5	33
Hot H_2O extract good timothy hay	10	70
Hot H_2O extract poor timothy hay	5	24
Hot H_2O extract poor timothy hay	10	71
Hot H_2O extract fresh-cut ladino clover	5	105
Hot H_2O extract fresh-cut ladino clover	10	98
Ground yellow corn, Hot H_2O extract	10	56
Ground corn cobs, Hot H_2O extract	10	23
Corn silage, Hot H_2O extract	10	79
Beef extract (Difco)	5	47
Yeast extract, dried (Difco)	1	94
Liver extract, Armour's, 1–20 dried	1	46
Dried brewers' yeast	1	87
Dried distillers' solubles	1	63
Dried distillers' solubles	2	83
Molasses, blackstrap	2	96
Soybean extract, "Soyalac" [3]	1	65

[1] Hot water extracts prepared by mixing finely ground material with 10 parts of water and the mixture brought to a boil. After filtering, the volume was adjusted to 1 to 10 with water, using the original weight of material and the filtrate was again brought to a boil, filtered, and the filtrate concentrated at room temperature under a fan.

[2] The extracts from the amount of material indicated were added to each flask.

[3] A product prepared by Loma Linda Foods—a spray dried water extract of soybean meal.

addition of 100 ml. of supernatant as compared with sediment alone was arbitrarily set at 100. The potency of other materials was calculated by the following method:

$$\text{Relative Activity} = \frac{\text{Treatment—Sediment}}{\text{Supernatant—Sediment}} \times 100$$

The values substituted in this expression were the percent cellulose digested in each flask. To illustrate, in a typical experiment, 1.5 gm. of cellulose was digested in the control flasks (with sediment) giving a

calculated relative activity of zero; whereas 7.0 gm. of cellulose was digested in flasks containing the same ingredients plus supernatant with a relative activity of 100.

The marked increase in activity (cellulose digestion) obtained from the addition of water extracts (boiling) of various plant materials or roughages clearly indicates that the basal medium is deficient in a factor(s) which is required by rumen microorganisms. Furthermore, the results show that alfalfa leaf meal, molasses, yeast, and rumen juice are especially good sources of the factor. It also seems of interest to note that 1–20 liver powder and a beef muscle extract (Difco) were not rich sources of activity. Although the experimental methods differ, indications that additional factors present in various natural materials were needed by rumen microorganisms *in vitro* have been reported by Hungate *et al.* (1950), Huhtanen *et al.* (1952), Doetsch *et al.* (1953), and more recently, by Ruf, Hale, and Burroughs (1953).

The effect on cellulose digestion of adding known compounds and alfalfa ash to the basal medium was also investigated. In table 3 the results of four experiments are reported. A combination of adenine, uracil, xanthine, nine B-vitamins, and molasses or alfalfa ash improved the rate of cellulose digestion to give a relative activity of 76 as compared to 100 for the supernatant addition to the basal medium. Further it was found that of the B-vitamins, biotin, paraminobenzoic acid (PABA), and vitamin B_{12} were responsible for the stimulation of microbial activity. A mixture of alfalfa ash, purines, and uracil or each of the materials alone was ineffective as a supplement to the basal medium. However, combinations of either ash or purines and uracil with the three-B-vitamins gave better results than was obtainable with B-vitamins alone. Apparently, in the absence of rumen juice, rumen microorganisms were unable to synthesize sufficient biotin, PABA, and vitamin B_{12} to satisfy their own metabolic needs for growth which in the case of cellulolytic organisms resulted in the retardation of cellulose digestion. These data would confirm the previous reports of Bentley *et al.* (1953) and Hall *et al.* (1953) in which they found that rumen microorganisms grown in a purified medium require additional growth factors which can be supplied in part by known compounds—certain B-vitamins appearing to be important.

Fractionation of Rumen Juice

In an effort to concentrate the factor present in rumen juice responsible for the increase in cellulose digestion, rumen juice was treated

with neutral lead acetate and the heavy white precipitate formed was removed by centrifugation. The excess lead was removed from the centrifugate with H_2S and centrifugation. A clear solution was thus

TABLE 3. THE EFFECT OF PURINES-PYRIMIDINE MIXTURE (P-P), B-VITAMINS, AND ALFALFA ASH ON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS *IN VITRO*

(48-hour experiments)

Additions to Basal Medium	Relative Activity			
	Experiment A	Experiment B	Experiment C	Experiment D
None	0	0	0	0
Supernatant	100	100	100	100
Aflalfa ash (0.5 gm.)			10	0
P-P [1]			37	
P-P + ash	4			
P-P + ash + B-vitamin solution, 1 ml.	17			
P-P + ash + B-vitamin solution, 5 ml.	30			
P-P + ash + B-vitamin solution, 10 ml.	59			
P-P + ash + B-vitamin solution, 20 ml.[2]	76			
P-P + ash + P.A., B_1, B_2, Niacin	4			
P-P + ash + F.A., B_6, PABA, Biotin, B_{12}	70			
P-P + F.A.[2]		13		
P-P + PABA		19		
P-P + Biotin		28		
P-P + B_6		0		
P-P + B_{12}		22		
P-P + Biotin, B_{12}, PABA		87		
Ash + PABA, B_{12}, Biotin			88	
P-P + PABA, B_{12}, Biotin			82	
PABA + B_{12} + Biotin			53	
P-P + Biotin and B_{12}			71	
P-P + Biotin and PABA			90	
P-P + B_{12} and PABA			55	
Biotin and PABA				61
Biotin and PABA + B_{12}				76

[1] Amount added, mg. per flask: Adenine, 20; Xanthine, 10; Uracil, 20.

[2] 20 ml. of B-vitamin solution contained the following amounts of each vitamin, μg per flask: Thiamine, 200; Riboflavin, 200; Calcium Pantothenate, 400; Niacin, 200; Pyridoxine, 100; Folic acid, 40; p-aminobenzoic acid (PABA), 200; Biotin, 20; vitamin B_{12}, 0.2. When the vitamins were used separately, the amounts as indicated above were used.

obtained. When the centrifugate itself or the dried residue of the centrifugate was assayed *in vitro* it was found that the cellulolytic factor was present in both preparations. The dried residue was extracted with absolute alcohol and the alcoholic extract was found to contain most of the activity present in the original residue. The alcohol extract

was first dried and redissolved in water before being tested. Further it was found that part of the materials responsible for the activity present in the alcoholic extract could be precipitated by the addition of 10 parts of ether to one part of the alcoholic extract. The activity of the ether precipitate represented only about one-half of the activity present in the original residue prepared from the rumen juice. The effect of these materials on cellulose digestion and the results of the statistical analyses are presented in table 4.

TABLE 4. THE EFFECT OF RUMEN JUICE FRACTIONS ON CELLULOSE DIGESTION *IN VITRO*

Additions to Basal Medium [1]	Pct. Cellulose Digestion, Av.
1. None (Sediment)	20.5 (8) [2, 3]
2. Supernatant (from rumen juice)	65.0 (8)
3. Filtrate from lead acetate ppt.	57.0 (6)
4. Abso. ETOH Ext. of dried lead acetate filtrate	53.0 (3)
5. Ether ppt. from abso. ETOH ext. of lead acetate filtrate	46.0 (7)
6. Ether-ETOH soluble portion from (5) above	31.0 (2)
7. 3 above treated with Norite A pH 3	8.5 (2)
8. 3 above treated with Norite A pH 4.5	8.0 (1)
9. 3 above treated with Norite A pH 5.0	22.0 (1)
10. 3 above treated with Norite A pH 6.0	37.5 (2)
11. 3 above treated with Norite A pH 9–11.0	40.5 (2)
12. 7, 8, 9 above eluted with KOH (0.1N)	39.0 (3)
13. 7 above eluted with NH_3 ETOH: H_2O	
$\cong$ 100 ml. supernatant	35.0 (1)
$\cong$ 200 ml. supernatant	36.0 (1)

[1] Unless otherwise stated, each fraction was equivalent to 100 ml. of supernatant.
[2] Number in parenthesis indicates number of experiments.
[3] L.S.D. 5% level 7.56; 1% level 10.6.

Further, in table 4, data are reported which show that the cellulolytic factors as obtained from the filtrate after treatment of the rumen juice with neutral lead acetate, can be adsorbed on Norite A (Pfanstiehl). An acidic water solution of the dried filtrate from the lead treatment (pH 3 to 5) which contained the equivalent of 100 ml. of rumen juice was mixed with 4 gm. of Norite A and stirred for 30 minutes. The cellulolytic factors were adsorbed on the norite A from acidic solutions while much less adsorption occurred from alkaline solutions. Part of this activity could be eluted with either dilute KOH solutions or a mixture of 5 parts of ethanol, 4 parts of water, and 1 part of NH_4OH. The latter eluent was easier to work with since after drying no undesirable salt residue was present which might interfere in the assay for

the factor *in vitro*. Some activity was lost during this treatment. This loss in activity appeared to be qualitative since doubling the amount of the eluate added to each flask did not increase the rate of cellulose digestion. Such data could be taken as an indication that there was selective elution of the factor(s) from the Norite A under the conditions used here but further investigations must be carried out before this can be stated with assurance.

TABLE 5. STABILITY OF THE ACTIVITY IN SUPERNATANT FROM RUMEN JUICE OR HOT WATER EXTRACT OF ALFALFA LEAF MEAL TO VARIOUS TREATMENTS

	Relative Activity	
	Supplement Used	
Treatment of Supplement to Basal Medium [1]	Alfalfa Leaf Meal Extract	Supernatant
None	100 [2]	100
Autoclave, 15 lb. 4 hr. pH 6.6	109	
Autoclave, 15 lb. 4 hr. 1 N HCL	78	
Autoclave, 15 lb. 4 hr. 1 N NaOH	104	
Autoclave, 15 lb. 24 hr. pH 7.0		121
Autoclave, 15 lb. 24 hr. 1 N HCL		0
Autoclave, 15 lb. 24 hr. 1 N NaOH		83
Ashing	0	
Lyophylization		86

[1] 100 ml. of supernatant or extract equivalent to 10 gm. of alfalfa leaf meal used in each treatment. The basal medium was used (see Table 1).
[2] The activity of unautoclaved alfalfa leaf meal extract was used as a reference.

The active factors in rumen juice or hot water extracts of alfalfa meal are stable to heat. Autoclaving at 15 lb. pressure for as long as 24 hours in neutral or alkaline (1 N NaOH) did not destroy the activity. However, some activity was lost after 4 hours autoclaving in 1 N HCL and after 24 hours autoclaving in the acid solution all the activity was destroyed. The results of the experiments concerned with stability are reported in table 5.

The cellulolytic factors as obtained from rumen juice in these experiments are soluble in butanol, propanol, methylcellusolve, water and ethanol and are relatively insoluble in acetone and ether.

The question arose as to whether the activity in rumen juice and/or the fractions prepared from rumen juice as described heretofore was

due to their B-vitamin content. Data presented in table 6 would indicate that there are additional cellulolytic factors in rumen juice.

From an inspection of the results it is immediately apparent that 100 ml. of supernatant was more active than any of the supplements used with the exception of mixtures in which the supernatant was also a component. It is also apparent that the combination of ash, xanthine,

TABLE 6. THE SYNERGISTIC EFFECT OF "CELLULOLYTIC FACTORS," B-VITAMINS, PURINES, PYRIMIDINE, AND ALFALFA ASH ON CELLULOSE DIGESTION *IN VITRO*

Additions to Basal Medium	Relative Activity Fermentation Time	
	36 Hours	48 Hours
None	0	0
Supernatant	100	100
Alfalfa ash, 0.5 gm.	0	0
Purine—Pyrimidine (P-P)[1]	5	6
Biotin, vitamin B_{12}, PABA, (B-vitamins)[2]	35	30
Ash + P-P	0	0
Ash + B-vitamins	22	52
B-vitamins + P-P	46	69
B-vitamins + P-P + ash	50	74
B-vitamins + P-P + ash + supernatant	114	112
Cellulolytic factors ≅ 100 ml. supernatant[3]	43	61
Cellulolytic factors + P-P	59	82
Cellulolytic factors + P-P + ash	55	79
Cellulolytic factors + P-P + ash + B-vitamins	68	83

[1] Purines and Pyrimidine (P-P) were added in the following amounts, mg. per flask: Adenine, 20; Xanthine, 10; Guanine, 20; Uracil, 20.

[2] The following amounts of B-vitamins used in these experiments, μg per flask: Biotin, 200; p-aminobenzoic acid, 500; and vitamin B_{12}, 0.2.

[3] Ether was added (10:1) to the absolute ethanol extract of dried filtrate from lead treatment of rumen juice and this precipitate was used in this experiment.

uracil, adenine, guanine, biotin, PABA, and vitamin B_{12} was more effective than any of the compounds alone. In fact, the purines, uracil, and ash together but without the B-vitamins were quite ineffective. With respect to factors from rumen juice, the addition of either B-vitamins and/or purines and uracil plus the cellulolytic factors from rumen juice *increased* cellulose digestion. In previous experiments it was found that increasing the level of B-vitamins above the level used in this experiment failed to improve cellulose digestion; thus, one could not explain the stimulatory effect of the vitamins when added in conjunction with the factor on the basis of merely supplying more vitamins.

A logical explanation appears to be that the various known substances and the factor act synergistically, perhaps promoting the synthesis of additional factors needed by the groups of organisms responsible for cellulose digestion in the laboratory rumen. Another possibility is that a change in the media permits the development of different groups of microorganisms.

The results of analyses for cellulose made on samples incubated 48 hours bring out another observation noted in experiments with rumen microorganisms grown *in vitro*. If the fermentation is allowed to go for 48 hours, cellulose digestion in flasks which contain the vitamins, purines, uracil, and ash tends to be relatively greater than at the end of 36 hours. This relationship has been observed in the flasks which contain only the sediment (control) as well. It would appear that as the fermentation period is lengthened bacterial growth factors needed by organisms which digest cellulose may be synthesized in the flasks, a condition which presumably exists in the rumen under natural conditions. If the fermentation time is reduced to 24 hours this effect is even more noticeable.

Briefly, the data in table 6 suggest that although various known substances enhance the rate of cellulose digestion there still appear to be factors in rumen juice, the identity of which are not known, needed by the rumen microflora for rapid growth as indicated by cellulolytic activity.

Discussion

Following the ingestion of feed, the number of microorganisms in the rumen of healthy adult animals increases rapidly to a peak at about two hours after eating (Bortree *et al.*, 1946). During this period of rapid cellulose digestion, the demand for major nutrients, such as nitrogen and a carbon source and the nutrients which could be classed as growth stimulants, would be presumably at a high level. These nutrients are available either from the freshly ingested ration or from the rumen contents which would include rumen juice as is referred to in this paper. Thus, animals fed a complete ration would supposedly have available to them an abundant supply of at least the minor nutrients, meaning those nutrients such as vitamins, trace minerals, etc. However, if ruminants are maintained on rations low in these factors over extended periods, digestion in the rumen may be impaired because of a lack of these growth factors. How this interference occurs

is a matter of conjecture for the lack of a certain nutrient may have an adverse effect on only one group of organisms and may influence the microflora indirectly. An example of interest is the apparent direct effect of feeding a poor quality roughage to cattle on the level of rumen microorganisms activity, as determined by cellulose digestion (Bentley *et al.*, 1951) and vitamin synthesis (Hunt *et al.*, 1952).

Certain B-vitamins, purines, and pyrimidines are known to have an important role in cellular metabolism and in the synthesis of new cells and proteins. That the availability of these compounds was a limiting factor in rumen microorganism activity as studied *in vitro* was demonstrated only after the cells had been separated from their natural source of these nutrients—rumen juice. Apparently synthesis of these stimulatory compounds from simple nutrients—ammonia, salts, and a carbon source—was inadequate to meet the needs of the microbial population. As a result the addition of supplements of biotin, PABA, vitamin B_{12}, along with purine and pyrimidine bases greatly enhanced the activity in the flasks as indicated by the rate of cellulose digestion. In addition to the known compounds tested in the experiments described herein, there is a factor or factors in rumen juice which will promote the development of the rumen culture *in vitro;* again as determined by an increase in the rate of cellulose digestion. Ruf *et al.* (1953) found that lambs fed a source of "microorganism-stimulating" factor improved appetite and increased the live-weight gains when used to supplement a purified type diet. To what extent *in vitro* observations are apropos in practice awaits further growth studies with lambs or steers fed rations in which the level of cellulolytic factor activity is critical.

Supplements designed to balance the nutritional deficiencies of poor quality roughages (Klosterman *et al.*, 1953; Beeson and Perry, 1953) have been of considerable interest in the practical feeding of cattle. Better live-weight gains were obtained with steers fed alfalfa leaf meal in place of soybean meal when the fattening type ration used included a poor quality timothy hay (Klosterman *et al.*, 1953). It seems probable that the results of *in vitro* experiments may be helpful in arriving at an explanation of feed lot results as referred to in this paper as well as offering an explanation for the generally accepted fact that cattle and sheep thrive on good roughage, whereas, despite the use of protein, mineral, vitamin A, and energy supplements, the performance of animals fed poor roughage feeds is often below expectations. This concept seems to be worthy of further study.

Summary

In vitro studies, in which rumen microorganisms separated from the juice by centrifugation were used as the inoculum, were carried out for the purpose of investigating the nutritional requirements of these organisms. The rate of cellulose digestion was used as a measure of activity.

The addition of autoclaved rumen juice, extracts of various plant materials, molasses, or yeast extract markedly increased the rate of cellulose digestion.

Biotin, vitamin B_{12}, para-aminobenzoic acid, xanthine, uracil, guanine, adenine, when added to the basal medium improved cellulose digestion. However, the amount of cellulose digested was always less than that digested in flasks which contained rumen juice supernatant, a water extract of alfalfa, yeast, or molasses. This observation was taken to indicate that undetermined factors were present in these natural products which when added to the *in vitro* fermenation enhanced the microbial activity.

An active substance(s) was concentrated from rumen juice by lead acetate precipitation followed by alcohol extraction of the dried filtrate residue. This material could be adsorbed on Norite A and eluted but the eluate was less active than the original rumen juice. The addition of B-vitamins, purines, and uracil to the flasks along with the eluate fraction increased the rate of cellulose digestion above the level obtained with the eluate alone. This would indicate that the factor was not one of these known compounds found to be active for rumen microorganisms *in vitro*.

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UTILIZATION OF UREA AND PROTEIN NITROGEN BY RUMINANTS FED HIGH-MOLASSES AND SUGAR RATIONS

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IT is generally recognized that, for ruminants, urea nitrogen in rations containing adequate energy and a certain level of preformed protein has a biological value approaching that of the proteins of many feeds (Hamilton *et al.*, 1948; Reid, 1953). However, in a recent comparison of different carbohydrate feeds as energy sources, evidence of inefficient utilization of urea nitrogen was obtained with rations containing a high percentage of cane molasses (Bell *et al.*, 1953). It appeared questionable whether in such rations the nitrogen of conventional protein supplements would be utilized to better advantage than that of urea (Briggs and Heller, 1943). Also, the results led to the question of the effect of different sugars on nitrogen utilization.

An opportunity to compare urea with a common protein supplement (soybean oil meal) in high-molasses rations was made available during a recent investigation of protein deficiency in beef cattle being fed rations based on prairie hay and molasses. In a separate study, the effect of sucrose, glucose and lactose on nitrogen utilization was investigated with sheep. Results of both these studies are presented at this time.

Experimental

In the comparison of urea with soybean oil meal, two 20-day nitrogen balance trials were conducted with six Hereford steers weighing between 650 and 700 lb. Two of the steers were fed a low-protein, high-molasses basal ration composed of 53.7 percent prairie hay, 44.8 percent cane molasses and 1.5 percent salt and bone meal mix (1:1.5). Two steers were fed urea as a supplement to the basal ration, and two were fed soybean oil meal. The urea ration was made by addition of approximately 2 percent of urea [3] to the molasses of the basal ration. In this

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[3] DuPont "Two-Sixty-Two", a commercial preparation of urea containing 42 percent nitrogen.

ration, 54 percent of the total nitrogen was derived from urea. The soybean ration was made by replacement of molasses with soybean oil meal in such amount that the total nitrogen content of the ration was equal to that of the urea ration. Molasses made up 30 percent of this ration, and oil meal 13 percent. The composition of the three rations as determined by proximate analysis is shown in table 1.

The first trial began 30 days after the steers were put on their respective rations and the second one 35 days later. Between trials the steers were kept on these rations and fed essentially equal amounts of dry matter, the level of feeding being determined by the maximum amount of feed consumed by the steers fed the low-protein ration.

TABLE 1. PERCENTAGE COMPOSITION OF HIGH-MOLASSES RATIONS FED TO STEERS (DRY MATTER BASIS)

Trial and Ration Designation	Organic Matter	Protein (N × 6.25)	Ether Extract	Crude Fiber	N-free Extract
Trial 1					
Basal	88.4	5.3	1.1	20.6	61.4
Urea	88.6	11.3	1.1	20.1	59.7
Soybean	89.7	11.2	1.7	21.2	55.6
Trial 2					
Basal	87.7	4.9	1.2	21.0	60.6
Urea	87.9	10.5	1.2	20.6	59.3
Soybean	88.7	11.0	1.8	21.7	54.2

During trials, feed intake was held constant with one exception; during the latter part of trial 2, both steers fed the basal ration refused part of the hay allowance. The manner of conducting the trials was the same as described in a previous paper (Bell *et al.*, 1953).

The utilization of nitrogen in rations containing different sugars as energy sources was determined in nitrogen balance trials with 12 wether lambs weighing between 60 and 80 lb. The experimental rations, which were identical except for the kind of sugar used, were composed of prairie hay, 50 percent; sugar (sucrose, glucose or lactose), 32; soybean oil meal, 16; and minerals,[4] 2. The average chemical composition of the rations on a dry matter basis was protein, 9.9 percent; ether-extract, 1.7; crude fiber, 16.6; nitrogen-free extract, 65.2; and ash, 6.6.

All the lambs were fed equal amounts of feed twice daily during

[4] The mineral mixture was salt, 42.8 percent; bone meal, 46.5; sodium sulfate, 8.6; and trace minerals (iron, copper, cobalt, manganese and iodine), 2.1.

20-day balance trials. As a matter of convenience in making feces and urine collections during these trials, the lambs were handled in two comparable groups, the collection period for one group corresponding with the preliminary feeding period of the other group. During a typical trial, two lambs in each group were fed the sucrose ration, two were fed the glucose ration, and two were fed the lactose ration. The lambs were changed from one ration to another and the trials repeated

TABLE 2. RESULTS OF DIGESTION AND NITROGEN BALANCE [1] TRIALS WITH STEERS FED HIGH-MOLASSES RATIONS SUPPLEMENTED WITH UREA AND SOYBEAN OIL MEAL

Trial	1			2		
Rations compared	Basal	Urea	Soybean	Basal	Urea	Soybean
Protein content (N × 6.25), percent [2]	5.3	11.3	11.2	4.9	10.5	11.0
Weight of steers, lb.	660	664	690	650	666	700
Daily dry matter intake,[3] gm.	4175	4275	4215	4312	4702	4749
Nitrogen intake, gm.	35.1	77.0	75.1	34.4	80.0	83.9
Nitrogen absorbed, gm.	10.0	50.4	43.7	7.8	50.4	48.9
Nitrogen in urine, gm.	13.9	39.6	34.4	12.7	37.1	34.4
Nitrogen retained, gm.	—3.9	10.8	9.3	—4.9	13.3	14.5
As percent of intake	—	14.0	12.5	—	16.6	17.3
As percent of absorbed	—	21.2	20.8	—	26.4	29.6
True digestibility of nitrogen, %	94.6	96.3	89.4	92.2	95.6	89.7
Biological value of nitrogen [4]	—	57.6	61.2	—	62.4	65.5
Apparent digestibility, %						
Organic matter	65.3	70.6	69.7	62.4	68.3	68.9
Protein	28.5	65.5	58.2	22.7	63.0	58.2
Crude fiber	59.5	69.7	72.8	50.5	67.2	71.9

[1] Nitrogen balance data on a daily basis.
[2] Dry matter basis.
[3] During trial 2, steers on the basal and urea rations refused part of the hay allowance.
[4] Estimated on the basis of metabolic nitrogen = 5.3 gm. per kg. dry matter intake, and endogenous nitrogen = $0.712W^{0.42}$, body weight (W) being expressed in kg. (Swanson and Herman, 1943).

in this manner until all lambs had received each ration. Feces and urine were collected during the last 10 days of each trial.

Results and Discussion

Nitrogen balance and digestibility data obtained with steers fed the high-molasses basal ration alone, and supplemented with urea and soybean oil meal, are shown in table 2.

The basal ration, which supplied about 35 gm. of nitrogen per day, failed to maintain the steers in positive nitrogen balance. Supplementation with urea in an amount to provide 77 to 80 gm. of nitrogen per day

increased nitrogen retention to 10.8 gm. in trial 1 and 13.3 gm. in trial 2. Likewise, supplementation with soybean oil meal increased retention to 9.3 gm. in trial 1 and 14.5 gm. in trial 2. A comparison of these nitrogen retention values expressed either as a percent of the total nitrogen intake or as a percent of the absorbed (apparent digested) nitrogen indicated that the two supplemented rations had about equal value. With each ration, about 21 percent of the absorbed nitrogen was retained in trial 1 and between 26 and 30 percent in trial 2. Values very similar to these have been reported for sheep fed urea and soybean oil meal as supplements to a basal ration containing 20 percent corn molasses (Johnson *et al.*, 1942).

Nitrogen digestibility values were consistently higher for the urea ration than the soybean ration, the apparent digestibility of nitrogen in the two trials averaging 64.2 for the urea ration and 58.2 for the soybean ration. Other measures of nitrogen utilization favored the soybean ration. For example, the biological values expressing the percentage utilization of true digested nitrogen were 57.6 and 61.2 for the urea ration in trials 1 and 2, respectively, and 62.4 and 65.5 for the soybean ration. Although the latter values indicate that the nitrogen of soybean oil meal was utilized more efficiently than that of urea in these high-molasses rations, the data are insufficient to warrant a positive conclusion. The trend of digestibility and biological values suggests incomplete conversion of urea nitrogen to protein in the rumen, a conclusion reached by Mills *et al.* (1942) in feeding hay-molasses-urea rations to dairy calves, and by Harris *et al.* (1942) in feeding high levels of urea to steers. Under more favorable conditions, where cereal grains are included with molasses to supply less soluble carbohydrate and part of the nitrogen of the concentrate feed, urea and soybean oil meal have similar nitrogen value (Johnson *et al.*, 1942). The beneficial effect of moderate amounts of molasses in urea rations, as compared to the large amounts used in the present study, has been demonstrated by several investigators (Scott, 1953).

Differences between the two supplemented rations in digestibility of nutrients other than nitrogen were not large. Of particular significance were the increases in digestibility of organic matter and crude fiber effected by supplementation of the basal ration with urea. These increases were apparent in both trials and were of the same order as those effected by supplementation of the ration with soybean oil meal. Average digestibility of organic matter was 64 percent in the basal ration and 69 percent in the urea and soybean rations. Average crude

fiber digestibility, which was 55 percent in the basal ration, was 68 in the urea ration and 72 in the soybean ration. The lower molasses content of the latter ration could account, in part, for its higher digestibility of fiber (Johnson *et al.*, 1942).

This favorable effect of urea on fiber digestion was not observed in the previous study conducted by Bell *et al.* (1953) with prairie hay and molasses rations. The apparent discrepancy between the two experiments can probably be explained by the difference in protein content of the basal rations. Many of the basal rations used by Bell *et al.* (1953) contained over 8 percent protein, as compared with 5 percent in the basal ration of the present experiment. In earlier experiments, urea increased the digestibility of fiber of prairie hay in rations containing from 6 to 7 percent protein (Gallup, Pope and Whitehair, 1952) but not in those containing over 7 percent (Dinning *et al.*, 1949). However, it consistently increased the digestibility of fiber of cotton seed hulls in rations containing as much as 8.5 percent protein (Gallup, Pope and Whitehair, 1952a).

Over a period of 3 months, the steers fed the basal ration lost an average of 60 lb. in weight while those fed essentially the same amount of dry matter with either urea or soybean oil meal as the nitrogen supplement maintained their weight. During the last 3 weeks of the period, blood values relating to the protein nutrition of the animals were as follows: Plasma protein, 6.3, 6.6 and 7.8 gm. per 100 ml. for the steers on the basal, urea and soybean rations, respectively; hemoglobin values in the same order were 11.1, 10.3 and 11.1 percent; and red cell volumes (hematocrit), 35, 34 and 36 percent. Initial plasma protein values were between 7.8 and 8.2 percent. The nutritional significance of the observed decrease in plasma protein of the steers on the basal and urea rations is a subject for further investigation.

Nitrogen balance and digestibility data obtained with lambs fed the rations containing different sugars are presented in table 3. Because of an occasional feed refusal or accident during collection periods, results are presented for only 11 of the 12 lambs fed each ration. Palatability differences between the rations were not apparent.

Average nitrogen retentions, expressed as percent of total nitrogen intake, for lambs fed sucrose, glucose and lactose were 15.4, 12.5 and 13.1, respectively; biological values in the same order were 64.7, 59.7 and 62.5 percent. All of these values are within the range frequently reported for the nitrogen of conventional rations fed to lambs. Differences between corresponding values were not statistically significant.

Average digestibilities of all nutrients were highest in the glucose

ration; however, differences in these values between rations were not statistically significant. Crude fiber digestibility, which is usually depressed by sugar supplements, was 55.1 percent in the glucose ration and 51 to 52 percent in the other two rations. Protein digestibility in the three rations varied from 57 to 61 percent. The results afford no positive evidence of one sugar affecting nitrogen utilization or nutrient digestibility in a manner different from another.

TABLE 3. RESULTS OF DIGESTION AND NITROGEN BALANCE[1] TRIALS WITH LAMBS FED SUCROSE, GLUCOSE AND LACTOSE RATIONS

Rations compared [2]	Sucrose	Glucose	Lactose
Protein content (N × 6.25), percent [3]	9.9	9.9	9.9
Number of lambs	11	11	11
Weight of lambs, lb.	69	68	67
Daily dry matter intake, gm.	657	654	656
Nitrogen intake, gm.	10.40	10.38	10.39
Nitrogen absorbed, gm.	6.09	6.32	5.94
Nitrogen in urine, gm.	4.49	5.02	4.59
Nitrogen retained, gm.	1.60	1.30	1.35
As percent of intake	15.4	12.5	13.1
Biological value of nitrogen [4]	64.7	59.7	62.5
Apparent digestibility, percent			
Organic matter	70.5	72.0	69.7
Protein	58.6	60.9	57.3
Ether extract	41.5	46.9	41.6
Crude fiber	52.1	55.1	50.8
N-free extract	77.8	78.7	77.2

[1] Nitrogen balance data on a daily basis.
[2] Sugar comprised 33 percent of each ration, the other ingredients being hay, soybean oil meal and minerals.
[3] Dry matter basis.
[4] Estimated on the basis of metabolic nitrogen = 0.55 gm. per 100 gm. dry matter intake, and endogenous nitrogen = 0.033 gm. per kg. body weight (Harris and Mitchell, 1941).

Summary

Nitrogen balance trials were conducted with six Hereford steers to determine the comparative value of urea and soybean oil meal as nitrogen supplements in high-molasses rations. The basal ration, which was composed of about equal parts prairie hay and molasses supplemented with bone meal and salt, contained 5 percent protein, dry matter basis. The two supplemented rations, one containing 2 percent urea and the other containing 13 percent soybean oil meal, supplied 11 percent protein. Each ration was fed in equal amounts to two steers.

Steers fed the basal ration lost an average of 60 lb. over a period of 3 months and were in negative nitrogen balance during balance trials. Those fed the supplemented rations maintained their weight and retained similar amounts of nitrogen, 13 to 17 percent of the intake.

Estimated biological values from 57.6 to 65.5 for the supplemented rations indicated more efficient utilization of the soybean oil meal nitrogen, but the data were insufficient to warrant a positive conclusion. Both supplements increased the digestibility of nutrients in the basal ration, crude fiber digestion being increased from an average value of 55 percent in the basal to 68 percent in the urea ration and 72 percent in the soybean ration.

The possible effect of different sugars on nitrogen utilization and digestion was studied in balance trials with 12 lambs fed 32 percent sucrose, glucose and lactose in separate but otherwise identical rations containing soybean oil meal as the protein supplement. Nitrogen retentions, biological values and nutrient digestibilities obtained with 11 lambs on each ration revealed no significant differences between the rations.

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NEW NITROGEN FEED COMPOUNDS FOR RUMINANTS—A LABORATORY EVALUATION

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A RUMINANT by virtue of the microflora in its spacious rumen is capable of utilizing simple nitrogen compounds to satisfy its protein requirements (Loosli *et al.* 1949). This is in addition to its ability to efficiently utilize the cellulosic material in the diet and to synthesize the required water soluble vitamins.

Although the ruminant can utilize nonprotein nitrogen compounds, urea is the principal material in this category that is now being used commercially in the ruminant feeds. In the past, ammonium bicarbonate and phosphate have been used in feeds but these have been unsatisfactory for one reason or another. Urea is particularly suited as a feed ingredient since it is an economical, odorless material of high nitrogen content and biological availability. The amount that can be used in a feed is limited somewhat by the rapid rate at which it can be converted to ammonia in the rumen. Other nonprotein nitrogen compounds which might not have this limitation were investigated as possible substitutes for urea. The artificial rumen technique was used for this purpose since it permitted rapid screening in the laboratory with small amounts of the experimental materials.

Experimental

The artificial rumen technique employed in this study was similar to that described by Burroughs *et al.* (1950). Each artificial rumen or fermentation flask (1 liter) was charged with 9 gm. of purified wood cellulose ("Solka Floc" SW 40A), 1 gm. of dextrose, 1 gm. of starch, and 0.6 gm. of cane molasses ash. The latter serves as a source of trace minerals. A nutrient salt solution approximating the composition of sheep saliva (McDougall, 1949) was also added, which contributed the following to each flask:

NaH_2PO_4	2.09 gm.
$NaHCO_3$	1.84 gm.
KCl	0.263 gm.
NaCl	0.263 gm.
$MgSO_4$	0.079 gm.
$CaCl_2$	0.027 gm.
$FeSO_4$	0.006 gm.

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The experimental nitrogen material equivalent to 187 mg. of nitrogen was then added to this mixture of nutrients, after which each flask was inoculated with 450 ml. of strained steer rumen contents collected before the morning feeding. The volume of each flask was then diluted to 900 ml. with distilled water. The flasks were placed in a 39° C. constant temperature bath and carbon dioxide bubbled through the system to assure the necessary anaerobic conditions. The pH of fermentation was maintained between 6.2 and 6.9 with 4N aqueous Na_2CO_3. At designated intervals during each fermentation period representative samples were removed for analysis of ammonia and urea by methods of the Association of Official Agricultural Chemists (A.O.A.C.) and cellulose by the method of Crampton and Maynard (1938). At the end of 24 hours the volume of the flasks was adjusted with distilled water to 900 ml., of which 450 ml. was used as inoculum for another flask containing new nutrients. This subculturing was repeated for four successive 24-hour periods.

Each series of fermentations had two controls: (1), a positive control which contained urea at a nitrogen level equivalent to that of the experimental compounds in the other flasks; and, (2), a negative control which received no added nitrogenous material throughout the fermentation period. The only nitrogenous material in the negative control originated from the rumen liquid used as inoculum on the first day.

In view of the exploratory nature of this study, the majority of data shown in the tables represent individual tests of each material over the 4-day fermentation period. The extent of nitrogen fixation in systems containing urea or ammonium salts was calculated on the basis of the diminution of urea and ammonia determined analytically (Burroughs, *et al.*, 1951). With a majority of test compounds, it was necessary to use the cellulose digestion data as an index of nitrogen utilization or availability, which is believed valid based on the findings in other studies (Belasco, 1953).

Results and Discussion

In order to tabulate the data from the independent experiments and thus permit comparison of the metabolic responses of the various candidate nitrogen compounds in a summary fashion, it was necessary to express the results as "percent of the urea response." This was a simple matter, since each individual experiment had positive (urea) and negative (no nitrogen compound added) controls. The difference

between the cellulose digestion and the difference between the ammonia nitrogen utilization values obtained in the positive and negative control flasks were considered as the true or 100 percent urea response. The cellulose digestion noted in the negative control flask was due to the nitrogenous materials, i.e., proteins, amino acids, ammonium salts, etc., present in the rumen liquid used as the inoculum on the first day of fermentation.

1. *Urea Derivatives*

This class of materials was first considered in the hope that such compounds would release ammonia at a slower rate than urea. It was thought that the bacterial enzyme systems in the rumen might possibly

TABLE 1. METABOLIC RESPONSE TO SIMPLE UREA DERIVATIVES

Compound	Cellulose Digestion (% of the urea response)	Bacterial Growth
Methylenediurea	16	Limited
n-Butylurea	11	Poor
Biuret	7	Poor
Hydrazoformamide	6	Poor
Ethylurea	5	Poor
Ethylenediurea	3	Poor
Acetylurea	—3[1]	Poor
Methylurea	—5[1]	Poor
Allylurea	—12[1]	Poor
Ethyleneurea	—14[1]	Poor
Dimethylolurea	—48[1]	None
Semicarbazide HCl	—70[1]	None

[1] Negative values indicate cellulose digestion rates lower than that of negative control which received no added nitrogenous compounds.

effect a hydrolysis of these compounds to urea and/or ammonia at a rate comparable with the rate of ammonia utilization by the bacteria. In these experiments, cellulose digestion was utilized as an index of nitrogen availability. The results shown in table 1 indicate that of all of the urea derivatives tested, only methylenediurea consistently showed a low level of available nitrogen. This may be due to its ability to hydrolyze under the slightly acidic conditions in the rumen to yield some free urea and polymethyleneurea. Biuret, n-butylurea, hydrazoformamide, ethylenediurea, ethylurea, acetylurea and methylurea all exhibited little or no available nitrogen. On the other hand, allylurea, ethyleneurea, and especially dimethylolurea and semicarbazide

hydrochloride showed toxic effects on the rumen microflora at the levels tested as noted by rates of cellulose digestion which were much less than that obtained in the negative control flasks. In addition, no bacterial growth was visible by the end of the fourth day.

2. Amides

The rumen bacteria appear to have sufficient amidase to hydrolyze the amides of monocarboxylic acids, such as formamide, acetamide,

TABLE 2. METABOLIC RESPONSE TO AMIDES

Compound	Av. Ammonia Level after 24 Hours (mg.)	Cellulose Digestion (% of the urea response)	Bacterial Growth
Urea	90–205[1]	100	Excellent
Propionamide	18	92	Good
Butyramide	48	72	Good
Glycinamide	—	54	Good
Formamide	75	51	Good
Acetamide	70	32	Good
Hydroxyacetamide	15	7	Poor
Succinimide	36	0	Poor
Oxamide	43	—9[2]	Poor
Malonamide	2	4	Poor
Glutaramide	11	2	Poor
Diglycolamide	16	4	Poor
Adipamide	7	2	Poor
Cyanoacetamide	18	—7[2]	Poor

[1] These values represent a range of the ammonia levels noted in flasks receiving urea (400 mg. per day).

[2] Negative values indicate a cellulose digestion rate lower than that of the negative control which received no added nitrogenous compound.

propionamide, butyramide and glycinamide to ammonia and the corresponding acid as indicated in table 2. These materials all provided the desired feature of a low ammonia level, but the resulting cellulose digestion was not as good as that obtained with urea. The low levels of free ammonia produced from these amides indicated a hydrolysis rate comparable to the rate of bacterial ammonia utilization.

Of interest in this connection were the experiments on glycinamide, which showed a higher nitrogen availability than acetamide on an equivalent nitrogen basis. In control experiments, glycine was included as the amino nitrogen control with acetamide as the amide nitrogen control. Surprisingly enough, glycine proved unavailable to the microflora as a nitrogen source, even though it is a naturally occurring

amino acid. Yet its amide, glycinamide, showed a higher nitrogen availability than acetamide.

In contrast to the amides of the monocarboxylic acids, the diamides of oxalic, malonic, glutaric, diglycolic and adipic acids appeared inert as nitrogen sources. One of the differentiating properties, which may account for this difference in activity, is water solubility; the diamides are quite water insoluble. The other related compounds which showed little or no nitrogen availability include hydroxyacetamide, succinimide, thioacetamide and cyanoacetamide.

TABLE 3. METABOLIC RESPONSE TO AMIDINES

Compound	Av. Ammonia Level after 24 Hours (mg.)	Cellulose Digestion (% of the urea response)	Bacterial Growth
Urea	90–205[1]	100	Excellent
Guanidine acetate	20	100	Excellent
Guanidine carbonate	30	90	Excellent
Guanidine hydrochloride	30	81	Excellent
Creatinine	57	68	Good
Creatine	27	61	Good
Dicyandiamide	15	2	Poor
Acetamidine	40	−1[2]	Poor
Guanylurea phosphate	25	0	Poor
Aminoguanidine bicarbonate	46	−7[2]	Poor
Aminoguanidine sulfate	20	−5[2]	Poor

[1] These values represent a range of the ammonia levels noted in flasks receiving urea (400 mg. per day).

[2] Negative values indicate a cellulose digestion rate lower than that of the negative control which received no added nitrogenous compound.

3. *Amidines*

Cellulose digestion data shown in table 3 indicate that guanidine hydrochloride, acetate and carbonate can serve as nitrogen sources for the rumen microflora. They have a distinct advantage over urea in the low levels of ammonia produced while supporting good bacterial growth and cellulose digestion. The low ammonia level noted with guanidine may be explained by a two-step enzymatic breakdown to yield maximum ammonia. The first step is assumed to be the hydrolysis of guanidine to ammonia and urea, which is followed by the hydrolysis of the resulting urea. Since the latter step is known to be rapid, it is reasonable to assume that the first step of this degradation is the limiting one, thus resulting in an overall slow release of ammonia and urea at a rate comparable to the rate of ammonia utilization by the bacteria.

Creatine and its inner anhydride, creatinine, also are good nitrogen sources. Both compounds gave the desired low ammonia level, indicating a similar two-step enzymatic hydrolysis to yield ammonia, carbon dioxide and sarcosine. Subsequent tests with sarcosine showed that its nitrogen is unavailable. This may explain the lower cellulose digestion level noted with creatine and creatinine as compared to urea on an equivalent total nitrogen basis.

Other amidines tested including acetamidine, aminoguanidine salts, and guanylurea phosphate all proved poor sources of nitrogen. Zeller *et al.* (1951) showed that arcaine and agmatine, both guanidine derivatives, can serve as bacterial nitrogen sources. Thus it seems that the structure $R_2N—C(=NH)NH_2$, where R is either hydrogen or an alkyl grouping, is susceptible to enzymatic hydrolysis to yield ammonia.

4. *Ammonium Salts*

The experiments with ammonium salts of various organic and inorganic acids point to the high availability of their nitrogen to the rumen microflora. In general, the main difference of the ammonium compounds as compared to the amides or amidines is the high level of ammonium ion noted when introduced into the system. However, an excess of ammonium salts may not be as hazardous as a similar excess of urea because of the accompanying acid radical.

a. *Inorganic Ammonium Salts.* Except for ammonium nitrate, the ammonium salts tested (table 4) all provided nitrogen for rumen bacterial growth and subsequent cellulose digestion. The free ammonia levels obtained with the ammonium salts wherever these were analytically possible were in the range of those noted for urea. Nitrogen utilization was also comparable with that obtained with urea. Similar data could not be accurately obtained in systems containing ammonium sulfamate, nitrilotrisulfonate and triamidodiphosphate due to the instability of these materials when subjected to the analytical conditions.

Ammonium nitrate proved toxic to the rumen microflora as indicated by microscopic examination of bacterial smears, high free ammonia levels and a cellulose digestion rate less than that observed in the negative control.

b. *Organic Ammonium Salts.* In tests with selected organic ammonium salts such as ammonium formate, succinate, lactate, alpha-ketoglutarate and possibly malate, the utilization rate of their nitrogen was higher than that noted with urea (table 4). Possibly the organic fragments of these compounds enter into some biosynthetic process of the rumen

TABLE 4. METABOLIC RESPONSE TO AMMONIUM SALTS

Compound	Av. ammonia Level after 24 Hours (mg.)	Ammonia Nitrogen Utilization (% of the urea response)	Cellulose Digestion (% of the urea response)	Bacterial Growth
Urea	90–205[1]	100	100	Excellent
Ammonium succinate	78	118	98	Excellent
Ammonium lactate	80	114	91	Excellent
Ammonium α-ketoglutarate	103	110	103	Excellent
Ammonium formate	102	107	104	Excellent
Ammonium malate	102	106	88	Excellent
Ammonium pyruvate	107	103	84	Excellent
Ammonium fumarate	95	102	100	Excellent
Ammonium citrate	137	100	94	Excellent
Ammonium adipate	143	97	91	Excellent
Ammonium acetate	95	95	97	Excellent
Ammonium sulfate	118	99	92	Excellent
Ammonium carbonate	164	91	97	Excellent
Ammonium sulfamate	...	..	78	Good
Ammonium nitriiotrisulfonate	...	..	87	Good
Ammonium triamidodiphosphate	...	..	67	Fair
Ammonium nitrate	397	0	—13[2]	None

[1] These values represent a range of ammonia level noted in all flasks receiving urea (400 mg. per day).

[2] A negative value indicates a cellulose digestion rate lower than that of the negative control which received no added nitrogenous compound.

TABLE 5. COMPARISON OF SELECTED AMMONIUM SALTS

Nutrient Variable	Av. Ammonia Level (mg.) after: 4 hr.	24 hr.	Av. Nitrogen Utilization (%) after: 4 hr.	24 hr.	Av. Cellulose Digestion (%)
No nitrogen (0 mg. N)	22	9	0	0	25
Urea (187 mg. N)	225	106	25	77	79
Ammonium formate (187 mg. N)	215	102	32	82	81
Ammonium lactate (187 mg. N)	181	80	59	87	74
Ammonium succinate (187 mg. N)	191	78	51	90	77
Ammonium sulfate (187 mg. N)	233	118	38	78	75

microflora that stimulates rapid nitrogen fixation. On this basis, some of the other salts of acids associated with the citric acid cycle, i.e., citrate, fumarate and pyruvate, were tried under similar conditions. All appeared comparable to urea, but lacked the stimulatory effect of some of the above ammonium salts. The cellulose digestion rates obtained with all the organic salts were comparable to that noted with urea.

TABLE 6. THE UTILIZATION OF AMMONIUM SUCCINATE IN COMPARISON WITH THAT OR UREA PLUS SODIUM SUCCINATE OR SUCCINIC ACID

	Average 24-Hour Data:		
Nutrient Variable	Ammonia Level (mg.)	Nitrogen Utilization (%)	Cellulose Digestion (%)
No nitrogen (0 mg. N)	7	0	18
Urea (187 mg. N)	127	71	61
Ammonium succinate (187 mg. N)	72	85	60
Urea + sodium succinate (187 mg. N)	114	73	58
Urea + succinic acid (187 mg. N)	114	76	60

Ammonium succinate and lactate were outstanding materials in that their nitrogen was utilized at a higher rate than that of urea, thus resulting in low levels of free ammonia. This is illustrated in table 5, where 4- and 24-hour data clearly indicate this phenomenon. Ammonium formate showed this same trend, but to a lesser degree.

Data shown in tables 6 and 7 again point to the rapid utilization of ammonium lactate and succinate nitrogen which could not be fully duplicated by the addition of energy to urea as glucose in the amount equivalent to the lactate of ammonium lactate. Nor could this effect be fully duplicated by the addition of equivalent amounts of sodium succinate or succinic acid to urea. It is possible that the supplemental succinate or succinic acid was metabolized before all the ammonia was available from the hydrolysis of urea (approximately two hours) and therefore could not affect the rate of nitrogen fixation. On the other hand, both ammonium succinate and lactate provided the maximum

ammonium ion concentration in the presence of the succinate or lactate fragments, and this may be the necessary condition for this stimulatory effect.

5. Miscellaneous Nitrogen Compounds

Two purines, uric acid and allantoin were found to be nitrogen sources for the rumen microflora. Even though allantoin, the ureide

TABLE 7. THE UTILIZATION OF AMMONIUM LACTATE IN COMPARISON WITH AMMONIUM CARBONATE OR UREA WITH ADDED GLUCOSE

	Average 24-Hour Data:		
Nutrient Variable	Ammonia Level (mg.)	Nitrogen Utilization (%)	Cellulose Digestion (%)
No nitrogen (0 mg. N)	14	0	25
Urea (187 mg. N)	140	67	65
Ammonium carbonate (187 mg. N)	164	61	64
Ammonium lactate (187 mg. N)	96	80	74
Urea plus glucose equivalent of lactate (187 mg. N)	123	73	62

of hydantoin, can serve as a nitrogen source, hydantoin itself is inert. This logically indicates that the enzymatic degradation of allantoin by the rumen bacteria follows the published pathway to allantoic acid, then to urea and glyoxal, and finally to ammonia, and not to urea and hydantoin.

The other types of nitrogen-bearing compounds tried which did not possess available nitrogen include ammeline, butylamine, caprolactam, cyanamide, cyanuric acid, dicyandiamide, ethylenediamine hydrochloride, hexamethylenetetramine, hydroxylamine hydrochloride, piperazine hexahydrate, pyrrole, sulfamide, thiourea, and uramil.

Summary and Conclusions

1. The data indicate the high availability of the nitrogen of numerous organic and inorganic ammonium salts. The use of ammonium formate,

alpha-ketoglutarate, malate and especially ammonium succinate and lactate showed higher rates of nitrogen utilization and lower free ammonia levels than urea and other ammonium salts tested. It is postulated that the organic fragments of these salts enter into some biosynthetic process stimulatory to nitrogen fixation by the rumen microflora.

2. The salts of guanidine, i.e., the carbonate, hydrochloride, and acetate as sole nitrogen sources in the artificial rumen gave low levels of free ammonia, and apparently provided an excellent nitrogen source for the growth of rumen microorganisms as indicated by high levels of cellulose digestion. Other amidines such as creatine and creatinine also proved effective nitrogen sources.

3. The amides of monocarboxylic acids also produce low levels of ammonia in addition to providing biologically available nitrogen as indicated by good bacterial growth. The cellulolytic response to these amides was generally less than that noted with urea. Those tested include formamide, acetamide, propionamide, n-butyramide and glycinamide. The diamides of dicarboxylic acids do not provide available nitrogen; those tested include oxamide, glutaramide, malonamide, diglycolamide and adipamide.

4. Uric acid and allantoin, examples of purines, also provide nitrogen for the rumen microflora.

5. The artificial rumen technique provides an *in vitro* approach capable of rapid screening of nonprotein compounds as candidate nitrogen feed materials.

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LEVELS OF PROTEIN IN PRACTICAL RATIONS FOR THE PIG

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IT has been reported recently that the protein requirements of swine are lower than indicated by the early studies of Carroll and Burroughs (1939) and Keith and Miller (1939, 1941). In particular, Catron *et al.* (1952) have studied the protein requirements of self-fed pigs from weaning to 75 lb., 75 to 150 lb., and 150 to 200 lb. live weight using an adequately supplemented corn-soybean oil meal ration. They reported that for the listed weight intervals, respectively, a 16–13–10 percent protein level combination supplied the pigs' need for protein when no antibiotic was fed; whereas, in the presence of the antibiotic a 14–11–8 percent level combination produced gains equivalent to higher levels of protein. However, the protein needs at the two heavier weight intervals were studied by arbitrarily reducing the protein fed by 3 percent from the previous feed level.

The investigations reported here were conducted to determine the minimum protein requirement of the pig from weaning to 100 lb. live weight and from 100 to 200 lb. live weight when practical-type rations formulated with yellow corn and soybean oil meal or menhaden fish meal were employed.

Experimental

Equal-Feeding Tests

The split-plot design as outlined by Snedecor (1946) was used for two experiments. In each experiment, from weaning to 100 lb., 32 pigs approximately 56 days of age were used; whereas, from 100 to 200 lb., 24 pigs were used. Durocs weighing an average of 42.0 lb. were used for the first experiment and Hampshires weighing an average of 36.0 lb. were used in the second experiment. From weaning to 100 lb., the 32 individuals were divided into eight outcome groups of four pigs each. Outcome groups were made up of littermates (or similar ancestry)

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on the basis of sex, thriftiness, and initial weight. Within the outcome groups, the pigs were assigned to the protein levels at random. Four of the groups chosen at random received soybean oil meal and the remaining groups were fed menhaden fish meal as the source of supplementary protein.

For the studies from 100 to 200 lb., pigs from the previous treatments were employed. As the pigs were removed from the early growth studies, outcome groups of three pigs each were formed. The individuals within an outcome group were assigned at random to different protein levels,

TABLE 1. PERCENTAGE COMPOSITION OF RATIONS FED

Ingredients	Corn-Soybean Oil Meal	Corn-Fish Meal
Solvent soybean oil meal (44.6% protein) and ground yellow corn[1, 2]	96.4	....
Menhaden fish meal (62.5% protein) and ground yellow corn[1, 2]	....	97.4
Mineral mixture No. 3[3]	2.0	2.0
Vitamin A and D oil (3000A-600D)	0.5	0.5
Steamed bone meal	1.0	....
Antibiotic-B_{12} feed supplement[4]	0.1	0.1
Vitamin mixture No. 36[5]	+	+

[1] The proportions were varied to yield the desired protein levels.

[2] By analysis (N x 6.25) the corn samples contained 7.4 percent protein in Experiment I, 8.5 percent protein in Experiment II, and 7.2 percent protein in Experiment III.

[3] The percentage composition of mineral mixture No. 3 was as follows: ground limestone, 40.0; steamed bone meal, 31.2; iodized NaCl, 25.0; $MgCO_3$, 2.0; $FeSO_4 \cdot H_2O$, 1.0; $MnSO_4 \cdot H_2O$, 0.56; $CoCl_2 \cdot 6H_2O$, 0.10; $CuSO_4$, 0.1 and $ZnCO_3$, 0.04.

[4] Guaranteed to contain 3 mg. of vitamin B_{12} (L. L. Elution Assay) and 2 gm. of procaine penicillin per pound.

[5] Additions to each pound of ration were as follows: Riboflavin, 1.4 mg.; calcium pantothenate, 5.0 mg.; niacin, 5.0 mg., and choline chloride powder (25 percent choline chloride), 1.0 gm.

and four of the groups were selected at random to receive the corn-soybean oil meal rations. The remaining groups received corn-fish meal rations.

The pigs were weighed at weekly intervals during the study and outcome groups were removed from the studies when the heaviest pig within a group attained the final weight.

The ingredients of the rations used are presented in table 1. In these two experiments the equalized-feeding technic as described by Mitchell and Beadles (1930) was used. All individuals within an outcome group received the same quantity of feed which was established at the amount consumed by the poorest eating pig. Feedings were made twice daily in individual feeding crates. Water was supplied *ad libitum* and the pigs were maintained in concrete pens at all times.

Full-Feeding Test

In a third experiment sixteen Hampshire pigs were allotted at random from outcome groups (grouped as above) to 10, 12, 14 and 16 percent protein rations formulated from corn and soybean oil meal. The pigs were managed in the same manner as described above, except full-feeding was followed and the pigs were fed to a final weight of 75 lb. The corn-soybean oil meal rations are presented in table 1.

TABLE 2. PERFORMANCE OF PIGS FED (EQUALIZED INTAKE) VARIOUS LEVELS OF PROTEIN FROM WEANING TO 100 POUNDS LIVEWEIGHT

Level of protein, percent	10.0	12.0	14.0	16.0	18.0
Experiment I					
Av. initial wt., lb.					
Corn-soybean oil meal	44.0	42.8	43.5	41.5	
Corn-fish meal	41.2	40.5	44.0	43.5	
Av.	42.6	41.6	43.8	42.5	
Av. daily gain, lb.					
Corn-soybean oil meal	0.90	0.99	1.07	1.08	
Corn-fish meal	0.65	0.79	0.81	0.86	
Av.	0.77	0.88	0.93	0.96	
Feed per lb. gain, lb.					
Corn-soybean oil meal	3.17	2.88	2.67	2.64	
Corn-fish meal	3.52	2.89	2.82	2.67	
Av.	3.33	2.88	2.74	2.65	
Experiment II					
Av. initial wt., lb.					
Corn-soybean oil meal		36.2	33.5	37.5	36.0
Corn-fish meal		35.2	38.0	36.5	36.0
Av.		35.7	35.8	37.0	36.0
Av. daily gain, lb.					
Corn-soybean oil meal		0.74	0.79	0.83	0.84
Corn-fish meal		0.71	0.82	0.89	0.88
Av.		0.73	0.81	0.86	0.86
Feed per lb. gain, lb.					
Corn-soybean oil meal		2.98	2.79	2.66	2.64
Corn-fish meal		3.21	2.77	2.57	2.59
Av.		3.09	2.78	2.62	2.62

Results

Initial to 100 Pounds Live Weight

The results of equally feeding rations containing various levels of protein to pigs from weaning (36.1 to 42.6 lb.) to 100 lb. live weight are presented in table 2.

According to the analysis of variance of Experiment I, the corn-soybean oil meal rations yielded daily gains superior (P less than 0.05) to corn-fish meal rations at all protein levels; however, it is believed that this effect merely reflects differences in feed consumption. With the technic employed here, the food consumption of all pigs within a quadruplet was controlled to that of the pig eating the least. The individuals at the 10 percent protein level consistently established the intake of the quadruplet, and those on fish meal ate considerably less than those on soybean oil meal. The supplements did not differ in Experiment II in which the food intake of all pigs within a quadruplet was generally established by the individual on the 12 percent protein ration.

The analysis of variance for each experiment revealed that the protein levels had a statistically significant effect (P less than 0.01) on the daily gains. In Experiment I the growth rate appeared to plateau at the 14 percent protein level with the pigs fed the corn-soybean oil meal rations. On the other hand, with fish meal as the supplementary protein there was an improved performance noted with each increment in protein. In Experiment II with fish meal, it was found that growth rate increased with each increase in protein up to 16 percent; the 16 percent protein level yielded essentially the same results as the 18 percent level. However, with soybean oil meal in that experiment the response to protein levels was not so clear cut. Poorest performance was obtained on the 12 percent level. At the 14 percent protein intake the rate of gain was slightly less than at the higher levels which were practically the same.

Since the pigs were equally fed, the feed efficiency, expressed as feed required per pound of gain, exhibited the same response to protein levels as the rate of gain.

A test of the homogeneity of the error variance for the two experiments indicated justification for pooling the data for the 12, 14, and 16 percent protein levels for further analysis. Some of the resulting means appear in table 3. The analysis indicated a significant variation (P less than 0.01) among the protein levels, but the interaction of supplement with protein levels was not significant. However, when the mean daily gains for the interaction of supplementary protein with protein levels were tested with the necessary difference (5%) values it was found that the slight difference between 14 and 16 percent protein levels on the corn-soybean oil meal ration was not statistically significant. All other

TABLE 3. THE EFFECT OF SUPPLEMENTARY PROTEIN SOURCE AND EXPERIMENT ON THE MEAN DAILY GAINS (POUNDS PER DAY) OF PIGS FED 12, 14, AND 16 PERCENT PROTEIN FROM 40 TO 100 POUNDS LIVEWEIGHT

	Protein level (percent)			Necessary Difference (P of 0.5)
	12	14	16	
Average	0.82	0.88	0.92	0.04
Supplement x protein level				
Soybean oil meal	0.87	0.93	0.95	0.06
Menhaden fish meal	0.76	0.83	0.89	0.06
Experiment x protein level				
Experiment I	0.90	0.95	0.98	0.06
Experiment II	0.73	0.81	0.86	0.06

comparisons of protein level within supplements were statistically significant.

In order to supply more convincing evidence on the performance of pigs fed various levels of protein in corn-soybean oil meal rations the results of the full-feeding study (Experiment III) are presented in table 4. When pigs were fed from approximately 40 to 75 lb. live weight 10 and 12 percent protein were inadequate; whereas, practically equal daily gains were noted on 14 and 16 percent protein. The analysis of variance indicated a significant difference (P less than 0.05) among the protein levels fed.

100 to 200 Pounds Live Weight

The results of equally feeding rations containing various levels of protein to pigs from 100 to 200 lb. live weight are presented in table 5.

TABLE 4. PERFORMANCE OF PIGS FULL-FED VARIOUS LEVELS OF PROTEIN IN A CORN-SOYBEAN OIL MEAL RATION FROM WEANING TO 75 POUNDS LIVE-WEIGHT (EXPERIMENT III)

Level of protein, percent	10	12	14	16
Number of pigs	4	4	4	4
Av. initial wt., lb.	38.0	42.5	39.8	42.5
Av. final wt., lb.	70.8	76.2	75.5	74.5
Av. daily gain, lb.	0.60	0.74	0.97	0.91
Av. daily feed intake, lb.	2.28	2.59	2.77	2.92
Feed per lb. gain, lb.	3.77	3.50	2.84	3.19

Analysis of variance indicated that neither protein level nor supplementary protein exerted an effect upon mean daily gains in Experiment I, although 12 percent protein was slightly superior to either 10 or 14 percent protein. The analysis of Experiment II showed that protein

TABLE 5. PERFORMANCE OF PIGS FED (EQUALIZED INTAKE) VARIOUS LEVELS OF PROTEIN FROM 100 TO 200 POUNDS LIVEWEIGHT

Level of protein, percent	8.0[1]	10.0	12.0	14.0
Experiment I				
Av. initial wt., lb.				
Corn-soybean oil meal		111.8	110.5	109.0
Corn-fish meal		102.0	99.5	101.5
Av.		106.9	105.0	105.2
Av. daily gain, lb.				
Corn-soybean oil meal		1.79	1.85	1.76
Corn-fish meal		1.75	1.83	1.80
Av.		1.77	1.84	1.78
Feed per lb. gain, lb.				
Corn-soybean oil meal		3.32	3.22	3.37
Corn-fish meal		3.22	3.06	3.15
Av.		3.27	3.14	3.25
Experiment II				
Av. initial wt., lb.				
Corn-soybean oil meal	100.0	101.8	99.8	
Corn-fish meal	96.2	93.8	94.2	
Av.	98.1	97.8	97.0	
Av. daily gain, lb.				
Corn-soybean oil meal	1.08	1.24	1.40	
Corn-fish meal	1.19	1.34	1.56	
Av.	1.13	1.29	1.48	
Feed per lb. gain, lb.				
Corn-soybean oil meal	4.39	3.82	3.38	
Corn-fish meal	4.24	3.75	3.22	
Av.	4.31	3.78	3.29	

[1] Rations without supplementary protein; they contained 8.88 percent protein by actual analysis (N x 6.25).

level exhibited a significant effect (P less than 0.01). There was an increase in daily gains noted with each increment in protein level, although the type of supplementary protein was without effect.

Since the error variances were not significantly different the data were pooled for the 10 and 12 percent protein levels in the two experiments. For the pooled data there was an effect for experiment, which is undoubtedly a result of differences in feed intake, and an effect for

protein levels; but the interaction of experiment with protein level was not significant. The superiority of 12 percent over 10 percent protein was a general effect in both experiments. However, when the mean daily gains for the interaction of experiment with protein level were tested with the necessary difference (5%) values, it may be noted in table 6 that 10 and 12 percent protein were not significantly different in Experiment I although they were different in Experiment II.

Discussion

In many of the studies reported here, maximum performance was not observed; however, the authors feel that the failure to obtain peak

TABLE 6. THE EFFECT OF SUPPLEMENTARY PROTEIN SOURCE AND EXPERIMENT ON THE MEAN DAILY GAINS (POUNDS PER DAY) OF PIGS FED 10 AND 12 PERCENT PROTEIN FROM 100 TO 200 POUNDS LIVEWEIGHT

Items	Protein level (percent) 10	12	Necessary Difference (P = 0.05)
Average	1.53	1.66	0.07
Supplement x protein level			
Soybean oil meal	1.52	1.63	0.09
Menhaden fish meal	1.55	1.71	0.09
Experiment x protein level			
Experiment I	1.77	1.84	0.09
Experiment II	1.29	1.48	0.09

performance does not impair the validity of the conclusions based on these data. Mitchell and Beadles (1952) have reported that valid determinations of protein requirements for maximum growth in the rat could be ascertained at a suboptimum performance. This principle has been verified for the pig by Becker *et al.* (1954).

With the corn-soybean oil meal ration, the studies here showed that the pig from 40 to 100 lb. required a minimum of 14 percent protein; whereas, from 100 to 200 lb. 12 percent protein was adequate. In general, these conclusions agree with those of Catron *et al.* (1952) who reported that the pig from weaning to 75 lb. required 14 percent protein in a corn-soybean oil meal ration; 12 percent for the heavier pig, however, is somewhat higher than the 11 and 8 percent they reported for the pig from 75 to 150 lb. and from 150 to 200 lb., respectively. There is some reason to believe that a lower protein level would be satisfactory for the pig when corn containing less than 8.5

percent protein is used. For instance, in Experiment I (7.4 percent protein corn) the difference between 10 and 12 percent protein was not statistically significant in the pig from 100 to 200 lb.

It was surprising that the pig required 16 percent protein in a corn-fish meal ration during the early growth period and 12 percent protein from 100 to 200 lb. Usually fish by-product proteins have been regarded as superior to plant proteins in supplementary value. Menhaden fish meal was inferior to soybean oil meal as a supplementary protein to corn when fed in 10 percent protein rations to pigs from 40 to 100 lb., but there was little or no difference for the heavier pig. When the supplementary protein sources were compared at adequate levels of protein for the pig from 40 to 100 lb. they were essentially equal. Since corn protein is notably deficient in lysine and tryptophan (Osborne and Mendel, 1914) and soybean protein is lacking in methionine (Mitchell and Smuts, 1932) the intakes of these amino acids were determined for the various rations. The results are presented in table 7. Compared to the corn-soybean oil meal ration, the fish meal rations were higher in lysine and methionine but lower in tryptophan at all protein levels. Hence, it is suggested that tryptophan is the limiting amino acid in the corn-fish meal rations.

The authors are impressed by at least two factors which are of importance in an expression of the protein requirements of swine fed practical rations formulated chiefly of yellow corn. The effect of the supplementary protein source has been mentioned and it is generally recognized as a significant element, but few attempts have been made to measure the magnitude of this influence. The protein content of the corn is also worthy of attention. Although the protein content of the corn samples used in these tests exhibited only a small variation, it is believed this variation produced differences in response to protein level. The amino acid values presented in table 7 support this belief. The 10 percent protein rations formulated with 7.4 percent corn contained only 65.5–68.1 percent corn protein in the total protein but with 8.5 percent protein corn 77.7–80.1 percent of the protein was from corn. Since corn protein is so markedly deficient in lysine and tryptophan, rations formulated with the higher protein corn were lower in lysine and tryptophan at all protein levels but particularly at the lower levels. It is noteworthy that the NRC (1947) reported that yellow corn samples in the United States contained from 6.57 to 10.93 percent protein (15% H_2O basis). Apparently this range does not include the high protein varieties referred to by Dobbins *et al.* (1951). Hence,

TABLE 7. THE CALCULATED DISTRIBUTION OF PROTEIN AND CERTAIN ESSENTIAL AMINO ACIDS IN THE RATIONS USED

Items		Percentage of Corn Protein in Total Protein	Percentage of Lysine[1] in Ration	Percentage of Tryptophan[1] in Ration	Percentage of Methionine[1] in Ration
Experiment I					
Yellow corn (7.4% protein)-soybean oil meal					
Percent protein	10	65.5	0.40	0.08	0.18
	12	51.2	0.52	0.10	0.20
	14	41.0	0.63	0.13	0.22
	16	33.5	0.75	0.16	0.24
Yellow corn (7.4% protein)-fish meal					
Percent protein	10	68.1	0.46	0.06	0.24
	12	54.0	0.61	0.08	0.30
	14	44.9	0.77	0.09	0.36
	16	37.5	0.93	0.11	0.42
Experiment II					
Yellow corn (8.5% protein)-soybean oil meal					
Percent protein	8[2]	100.0	0.25	0.04	0.17
	10	77.7	0.36	0.06	0.19
	12	60.8	0.48	0.09	0.21
	14	48.8	0.60	0.12	0.23
	16	39.7	0.72	0.15	0.25
	18	32.7	0.84	0.17	0.27
Yellow corn (8.5% protein)-fish meal					
Percent protein	8[2]	100.0	0.25	0.04	0.18
	10	80.1	0.39	0.05	0.23
	12	64.0	0.55	0.07	0.29
	14	52.7	0.71	0.09	0.35
	16	44.1	0.88	0.10	0.41
	18	37.5	1.04	0.12	0.47

[1] Values for ration ingredients determined by actual assay in Experiment I. In Experiment II the same values were used except for corn for which the values were adjusted for the added nitrogen content.

[2] Rations without supplementary protein; they contained 8.88 percent protein by actual analysis (N x 6.25).

protein feeding allowances must include a safety margin which allows for wide variations in the relative contribution of corn to the total protein and essential amino acids in the ration and also for wide variations in amino acid composition of the supplementary protein employed. Precise determinations of protein requirements for practical application seem rather futile.

In agreement with earlier studies by Becker *et al.* (1954) the responses noted here indicate that the amino acid requirements of the weanling pig must be lower than currently recognized. Rations containing 0.63 percent lysine, 0.13 percent tryptophan, and 0.22 percent methionine (no measurement of cystine) seemed adequate in these studies. In this regard, Pond *et al.* (1953) reported that the addition of 0.10 percent DL-lysine to a 12 percent protein corn-soybean oil meal ration was more effective than higher levels to improve growth rate and feed efficiency. In addition, Catron *et al.* (1953) reported that lysine did not improve a 14 percent corn-soybean oil meal ration, and that 0.10 percent L-lysine hydrochloride was superior to 0.05 percent but equal to greater additions to a 12 percent protein corn-soybean oil meal ration.

The 100-pound pig exhibited satisfactory performance on rations containing 0.52 percent lysine, 0.10 percent tryptophan, and 0.20 percent methionine (no measurement of cystine). However, in view of the satisfactory performance in Experiment I (7.4 percent protein corn) levels of 0.40 percent lysine, 0.08 percent tryptophan, and 0.18 percent methionine may be nearer to the minimum requirements.

Summary

The protein requirement of the pig at two stages of growth was determined primarily by the equalized feeding technic with practical-type rations containing an antibiotic. Under the conditions of these tests, the pig from 40 to 100 lb. live weight required a minimum of 14 percent protein in a corn-soybean oil meal ration and a minimum of 16 percent protein in a corn-menhaden fish meal ration. From 100 to 200 lb. live weight, the pig needed a minimum of 12 percent protein when either soybean oil meal or menhaden fish meal was used as the source of supplementary protein with yellow corn.

For the pig from 40 to 100 lb., menhaden fish meal was less satisfactory than soybean oil meal when fed in 10 percent protein rations, although these two supplements were of equal value in rations containing 18 percent protein.

In addition to the effect of the supplementary protein, the distribution of certain essential amino acids in the rations and the corresponding observed performance of the pigs, particularly on low protein rations from 100 to 200 lb., emphasize the importance of the protein level in the corn used in protein requirement studies.

Lysine, tryptophan, and methionine content of the rations which

supported satisfactory performance was 0.63, 0.13 and 0.23 percent, respectively, from 40 to 100 lb.; and 0.52, 0.10 and 0.21 percent, respectively, from 100 to 200 lb.

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SOME PLANT AND ANIMAL SOURCES OF CRUDE PROTEIN FOR WEANLING PIGS FED IN DRYLOT [1]

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SOYBEAN oil meal and meat and bone scrap are used extensively in swine rations as supplementary sources of protein and other nutrients, whereas dried skim milk is usually too expensive to permit wide-scale use. Studies of the effect of heat treatment and solvent upon the nutritive value of soybean oil meal for swine have been reported by Robison (1930, 1951), Dyer and Krider (1950), Terrill *et al.* (1951) and Becker *et al.* (1953). Smith and Maynard (1935) reported comparisons of powdered skim milk and tankage for pigs. Henson *et al.* (1952) and Bloss *et al.* (1953) demonstrated that tryptophan is the limiting amino acid of corn and tankage rations and corn and meat and bone scrap rations. Beeson *et al.* (1949) showed that tryptophan is an essential amino acid for the weanling pig. Shelton *et al.* (1951) reported the DL-tryptophan requirement of the weanling pig to be 0.2 percent in a 24.5-percent protein ration. Studies to re-evaluate the protein requirements of growing-fattening swine have been reported by Catron *et al.* (1952) and Hoefer *et al.* (1951).

The objective of this study was to evaluate some plant and animal sources of protein for weanling pigs fed in drylot.

Experimental Procedure

Experiment 1

In this group-feeding experiment, weanling pigs on concrete were fed from 36 to 100 pounds live weight. The objective was to compare sources of supplemental protein and levels of protein. The following sources of supplemental protein were tested in rations which contained ground yellow corn, vitamins, minerals and antibiotic: (a) blended

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[2] Present address: Chas. Pfizer & Co., Inc., Terre Haute, Indiana.

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extracted soybean oil meal, (b) a new-process soybean oil meal [1] and (c) meat-and-bone scrap with and without tryptophan.

Hampshire and crossbred Hampshire × Chester White pigs as they reached 56 days of age were allotted to the six experimental treatments on the basis of initial weight, litter and sex. The pigs had been raised

TABLE 1. PERCENTAGE COMPOSITION OF RATIONS IN EXPERIMENT 1

	Lot Number and Treatment					
Ingredients	1 Blended SBOM Ration (18% protein)	2 New-Process SBOM Ration (18% protein)	3 Blended SBOM Ration (16% protein)	4 New-Process SBOM Ration (16% protein)	5 Blended Meat and Bone Scrap Ration (18% protein)	6 (5) plus 0.1% DL-Trypto-phan (18% protein)
Ground yellow corn	70.7	72.5	75.9	77.7	76.72	76.72
Blended SBOM[1]	26.0	....	20.8	....	....	....
New-process SBOM[2]	....	24.2	....	19.0	....	....
Blended meat and bone scrap[3]	....	....	....	....	22.40	22.30
Steamed bone meal	1.0	1.0	1.0	1.0	....	....
Mineral mixture[4]	2.0	2.0	2.0	2.0	....	....
Viadex (4000A, 750D)	0.2	0.2	0.2	0.2	0.20	0.20
B-vitamins[5]	+	+	+	+	+	+
Vitamin B_{12} and antibiotic suppl.[6]	0.1	0.1	0.1	0.1	0.10	0.10
DL-tryptophan	....	....	....	....	....	0.10
Iodized salt	....	....	....	....	0.50	0.50
Mineral mixture[7]	....	....	....	....	0.08	0.08

[1] Regular solvent soybean oil meal blended from five sources.

[2] A solvent extracted soybean oil meal supplied by Central Soya Co., Inc., which contained by analysis 11.83% moisture, 48.02% crude protein, 1.62% fat, 4.53% fiber, 5.29% ash and 8.33% water-soluble protein.

[3] Meat-and-bone scrap blended from four sources.

[4] Mineral mixture contained 75 lb. iodized salt, 93 lb. steamed bone meal, 120 lb. ground limestone, 6 lb. magnesium carbonate, 3 lb. ferrous sulfate, 136 gm. copper sulfate, 763 gm. manganous sulfate, 136 gm. cobaltous chloride, 54 gm. zinc carbonate and 272 gm. potassium carbonate.

[5] B-vitamins added per lb. of ration were 1.5 mg. riboflavin, 5 mg. calcium pantothenate and 5 mg. niacin.

[6] Guaranteed to contain 12.5 mg. vitamin B_{12} (L.L.D. cup assay) and 2 gm. procaine penicillin per pound.

[7] Composition of mineral mixture (% or lb.): Magnesium carbonate 50.0, ferrous sulfate 25.0, potassium carbonate 5.0, copper sulfate 2.5, cobaltous chloride 2.5, manganese sulfate 14.0 and zinc carbonate 1.0.

by sows on rye pasture, and they had access to a pig starter ration in a creep during the suckling period. The experimental rations are given in table 1. The pigs were weighed at weekly intervals. Each pig was removed from test on the weigh day that its live weight was approximately 100 lb.

[1] A process described in patent No. 2585793 issued February 12, 1952 to Central Soya Co., Inc., Fort Wayne, Indiana for treating solvent (hexane) extracted soybean oil meal by supplying liquid to the meal, heating the meal below the boiling point of water, introducing steam into the meal and condensing the steam upon the meal to bring the moisture content of the meal to between 14 and 30%, followed by cooking at a temperature above the boiling point of water while keeping the meal in motion. The product is described in further detail in Pending Patent No. 211351.

Experiment 2

In this individual-feeding experiment the relative quality of protein in (a) blended extracted soybean oil meal, (b) new-process extracted soybean oil meal (c) blended meat-and-bone scrap and (d) dried skim milk was determined by the growth method proposed by Osborne *et al.* (1919) as modified for use with the pig by Geurin *et al.* (1951).

TABLE 2. PERCENTAGE COMPOSITION OF RATIONS IN EXPERIMENT 2

	Lot Number and Treatment			
	1	2	3	4
Ingredients	Blended SBOM	New Process SBOM	Blended Meat and Bone Scraps	Dried Skim Milk
Dried skim milk	....	.....	....	32.00
New-process soybean oil meal[1]	....	23.00	....	....
Blended soybean oil meal[2]	24.50	....	....	....
Blended meat and bone scraps[3]	....	....	22.00	....
Corn starch	48.43	49.59	52.00	43.44
Crude corn oil	2.26	2.27	0.30	2.12
Woodflock	0.56	0.64	1.52	1.94
Dextrose	21.75	22.00	23.18	18.00
Mineral mixture[4]	2.00	2.00	....	2.00
Vitamin A & D oil (4000A, 750D)	0.50	0.50	0.50	0.50
Iodized salt	....	....	0.50	....
Mineral mixture[5]	....	....	0.08	....
B-vitamins[6]	+	+	+	+
Procaine penicillin (5 mg./lb. ration)	+	+	+	+
Percent crude protein, by analysis	11.08	10.73	11.09	10.21

[1] See footnote 2, Table 1.
[2] See footnote 1, Table 1.
[3] See footnote 3, Table 1.
[4] See footnote 4, Table 1.
[5] See footnote 7, Table 1.
[6] B-vitamins added per lb. of ration were 0.5 mg. thiamine, 1.4 mg. riboflavin, 6.0 mg. niacin, 3.7 mg. calcium pantothenate, 0.6 mg. pyridoxine, 454 mg. choline chloride and 9 mcg. vitamin B_{12}.

The same samples of soybean oil meal and meat-and-bone scrap used in Experiment 1 were employed in Experiment 2.

Sixteen Chester White barrows averaging 68 days of age were divided into four blocks on the basis of litter, weight, general condition and thriftiness. Individuals within each block were then assigned at random to the four treatment groups. The rations were equated to approximately the same protein, fat and fiber content; they are presented in table 2. The pigs were hand-fed twice daily in individual

feeding stalls at as nearly full feed as possible. Water was available at all times. Daily feed and weekly live weights were recorded. The pigs were kept on concrete floors.

Results and Discussion

The results of Experiment 1 are summarized in table 3.

Because the individual pigs were on feed for various periods of time, the average daily gains were ascertained with different precisions,

TABLE 3. COMPARISON OF PLANT AND ANIMAL SOURCES OF CRUDE PROTEIN FOR WEANLING PIGS SELF-FED IN DRYLOT

(Experiment 1)

	Lot Number and Treatment					
	1	2	3	4	5	6
Items Compared	Blended SBOM Ration (18% protein)	New-Process SBOM Ration (18% protein)	Blended SBOM Ration (16% protein)	New-Process SBOM Ration (16% protein)	Blended Meat and Bone Scrap Ration (18% protein)	(5) Plus 0.1% DL-Trypto-phan (18% protein)
Number of pigs	8[1]	8[2]	9	9	8[2]	9
Av. initial wt., lb.	37	38	36	36	36	36
Av. final wt., lb.	100	100	101	102	100	100
Av. daily gain,[3] lb.	1.23	1.14	1.27	1.14	0.96	1.24
Av. daily feed per pig, lb.	3.61	3.45	3.82	3.50	3.95	4.21
Av. feed per lb. gain, lb.	2.99	3.08	3.07	3.11	4.17	3.33

[1] Does not include two unthrifty pigs removed during the test.
[2] Does not include one unthrifty pig removed during the test.
[3] Unadjusted.

necessitating statistical analysis by the method of fitting constants. In a number of instances two or more pigs from the same litter received the same treatment, so that there were 14 degrees of freedom for error. These were examined for an effect of initial weight, it being supposed that pigs which had gained better than average before the experiment were likely to gain better on the test, but this proved not to be the case.

The analysis consisted of fitting constants for protein source, protein level, tryptophan and litter. It was shown that there was no interaction of treatments with litters, no interaction of protein source with protein level, and no effect of increasing the protein level from 16 percent to

18 percent. The addition of 0.1 percent DL-tryptophan to the corn-and-meat-and-bone scrap ration increased the average daily gain by 0.29 pound, significant at the 1 percent level. Differences between the three protein sources (blended extracted soybean oil meal, new-process extracted soybean oil meal and meat-and-bone scrap) were significant, barely failing to reach the 1 percent level. The adjusted average daily gains in pounds were 1.19 for blended soybean oil meal, 1.09 for new-process soybean oil meal, 0.96 for meat-and-bone scrap and 1.24 for meat-and-bone scrap plus 0.1 percent DL-tryptophan.

The results of Experiment 1, with respect to the two protein levels tested, are in agreement with the results of Catron *et al.* (1952) for the same levels and agree with the results of Hoefer *et. al.* (1951) who tested nearly the same levels for pigs up to 100 pounds in weight.

The finding that a ration of corn and meat-and-bone scrap fortified with minerals, vitamins and antibiotic was improved significantly by the addition of 0.1 percent DL-tryptophan is in agreement with the results of Bloss *et al.* (1953) who fed a similar ration with no antibiotic and noted significant increases in gains with additions of 0.04 percent, 0.06 percent, or 0.12 percent DL-tryptophan. Bloss *et al.* (1953) further reported that the addition of the antibiotic oxytetracycline to a corn-and-meat-and-bone scrap ration to which no DL-tryptophan had been added failed to increase daily gains significantly.

In Experiment 2, the three protein sources tested in Experiment 1 and dried skim milk were compared in rations containing about 11 percent crude protein, the protein being furnished entirely by the protein source being tested. The results are summarized in tables 4 and 5.

Statistical analysis of the gains showed that dried skim milk exceeded new process soybean oil meal and that blended soybean oil meal exceeded meat-and-bone scrap at the 1 percent significance level, but new process soybean oil meal did not exceed blended soybean oil meal at the 5 percent level. For average daily feed consumption, dried skim milk exceeded new process soybean oil meal and blended soybean oil meal exceeded meat-and-bone scrap at the 5 percent level, but new process soybean oil meal did not exceed blended soybean oil meal at the 5 percent level. However, the differences in feed consumption were not sufficient to explain the differences in gains, the significance relations for gains being unchanged by covariance adjustment to equal feed consumption, though the differences were much reduced.

Thus, from the results of Experiment 2 and the fact that the two treatments involving soybean oil meal did not differ significantly, it

TABLE 4. RESULTS OF PROTEIN EFFICIENCY TEST (EXPERIMENT 2, INDIVIDUAL-FEEDING IN DRYLOT)

Items Compared	Treatment 1 Blended Soybean Oil Meal	2 New-Process Soybean Oil Meal	3 Blended Meat and Bone Scrap	4 Dried Skim Milk
Number of pigs	4	4	4	4
Av. initial wt., lb.	42	42	42	42
Av. final wt., lb.	74	79	54	95
Av. daily gain (unadjusted), lb.	0.88	1.01	0.40	1.43
Av. daily gain (adjusted),[1] lb.	0.90	0.98	0.55	1.23
Gain per lb. protein consumed, lb.	3.23	3.61	1.59	4.54
Av. daily feed per pig, lb.	2.45	2.62	1.88	3.08
Av. feed per lb. gain, lb.	2.79	2.58	5.66	2.16

[1] Covariance adjustment to equal feed consumption.

is apparent that the feed consumption and gains of pigs fed dried skim milk, soybean oil meal, or meat-and-bone scrap rations were altered significantly through some mechanism. After the gains were adjusted for variations in feed consumption, the growth responses to these sources of protein were still significantly different. Unless these protein sources brought about nutritional imbalances or interactions, or furnished growth factors not present in sufficient quantities in the remainder of the ration, one may assume that the amino acid balance or protein quality of the dried skim milk, soybean oil meal and meat-and-bone scrap was significantly different and in decreasing order of superiority as listed.

In the first experiment, the average gain on blended soybean oil meal exceeded that on the new-process meal, but the opposite occurred in the second experiment. This may reflect differences in the experimental conditions, the meals being tested as supplements to corn in rations

TABLE 5. ANALYSIS OF COVARIANCE OF FEED CONSUMPTION (x) AND WEIGHT GAIN (y) FOR EXPERIMENT 2

Source	SS and Products d/f	Sx^2	Sxy	Sy^2	Errors of Estimate d/f	Adj. Sy^2	M.S.	V.R.	P
Rations	3	4050.82	3685.79	3355.5	3	304.13	101.38	17.34	<0.001
Outcome groups	3	2801.99	646.96	169.0	3	30.27	10.09		
Error	9	1223.64	419.39	190.5	8	46.76	5.845		

containing 16 and 18 percent crude protein in the first experiment and as the sole source of protein in rations containing about 11 percent crude protein in the second experiment. The disagreement between the two experiments as to the relative value of these two meals does not reach the 5 percent significance level by the Behrens-Fisher test (Fisher and Yates, 1953).

Summary

In a growing-fattening test with pigs (weaning to 100 pounds live weight) in dry lot, the following protein sources were compared in rations containing ground yellow corn fortified with vitamins, minerals and antibiotic: (A) a blend of five samples of extracted soybean oil meal, (B) a new-process extracted soybean oil meal and (C) a blend of four samples of meat-and-bone scrap. Sources (A) and (B) were tested at both 16 and 18 percent levels of crude protein; source (C) was tested at 18 percent protein alone, and with the addition of 0.1 percent DL-tryptophan. The three protein sources were significantly different, barely failing to reach the 1 percent level. The addition of tryptophan to meat-and-bone scrap increased gains significantly (1 percent level). The adjusted average daily gains in pounds for the above treatments were: (A) 1.19, (B) 1.09, (C) 0.96 and (C plus 0.1 percent DL-tryptophan) 1.24. There was no significant interaction and there was no effect of increasing protein level from 16 to 18 percent.

In a protein efficiency test, semi-purified rations containing about 11 percent crude protein derived solely from the protein sources (A), (B) and (C) of the first experiment and from dried skim milk (D) were fed to weanling pigs in dry lot for 37 days. Statistical analysis of the gains showed that (D) exceeded (B) and (A) exceeded (C) at the 1 percent significance level, but (B) did not exceed (A) at the 5 percent level. For feed consumption, (D) exceeded (B) and (A) exceeded (C) at the 5 percent level, but (B) did not exceed (A), at the 5 percent level. The significance relations for gains was unchanged by covariance adjustment to equal feed consumption, although the differences were much reduced.

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OBSERVATIONS ON THE USE OF WASTE BEEF FAT IN SWINE RATIONS [1]

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NUTRITIONISTS have shown that a limited amount of fat is essential for animal growth and health. Studies by Evans and Burr (1927), Burr and Burr (1929), Deuel *et al.* (1950) and Cerecedo *et al.* (1952) have shown this vital need for fat in the rations of rats. Witz and Beeson (1951) working with weanling pigs on a purified ration low in fat reported typical external fat deficiency symptoms of dermatitis, loss of hair, unthriftiness and slow growth. Thus, it has been demonstrated that a source of dietary fat is necessary to supply the essential fatty acids, aid in the absorption of the fat soluble vitamins, and perhaps exert other beneficial effects, in addition to providing a source of energy.

Fats have generally been considered too expensive to be used as a source of energy in the rations of farm animals, but in recent years the prices of animal fats in comparison to grains have been such as to make a comparative feeding study desirable. This study was undertaken to ascertain the feeding value of raw ground beef fat and to determine its effect upon average daily gains, feed efficiency and certain carcass measurements. In addition, the effect of supplementing the waste fat ration with certain B-complex vitamins was studied.

Experimental Procedure

Animals

A total of four experiments was included in this study. In Experiment I purebred Duroc hogs averaging approximately 115 lb. were used. In Experiment II purebred Duroc and Hampshire hogs weighing approximately 120 lb. were used. In Experiment III purebred weanling Duroc and crossbred Duroc × Spotted Poland China pigs were used,

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while purebred Duroc, Hampshire and Duroc × Hampshire crossbred weanling pigs were used in Experiment IV. In each experiment the pigs were allotted as equally as possible on a basis of breed, sex and initial weight.

Experimental Rations

In Experiment I, Lot 1 received a basal corn-soybean oil meal-meat and bone scraps ration (table 1). Lot 2 received essentially the same ration except that 10 percent by weight of raw ground waste beef fat was substituted for corn, with the protein levels being equalized by making the necessary adjustment in the proportion of the protein supplement included. The fat was coarsely ground and mixed by hand. All lots were fed in concrete pens, which were washed daily. The pigs were weighed weekly and records were kept on gains and feed consumption. The rations used in Experiment II were identical to Experiment I (table 1).

In Experiment III, Lot 1 received a basal corn-soybean oil meal-meat and bone scraps ration (table 1) without B-vitamin supplementation. Lot 2 received a 10 percent waste beef fat ration in which the protein level was equalized to that of Lot 1. Lot 3 was fed the same ration as Lot 2 except it was supplemented with 7 B-vitamins. Lot 4 was self fed a 30 percent protein mixture and hand fed all the raw ground beef fat they would eat. All other lots were self fed. In Experiment IV, Lot 1 received the same basal ration as used for Lot 1 in Experiment III. Lot 2 was fed 10 percent waste beef fat supplemented with 7 B-vitamins. Lot 3 received the same ration except only thiamin, riboflavin and niacin were added. Lot 4 was fed a ration containing 15 percent waste beef fat to which 7 B-vitamins were added.

Slaughter Procedure

Experiment I was concluded when the lightest lot averaged 180 lb. live weight. The hogs were slaughtered by a local meat packer. Warm carcass weights were obtained immediately after slaughter; cooled carcass weights were taken after chilling at 36° F. for 24 hours. Carcass yields were calculated on the basis of live weight at the close of the experiment for both the warm and chilled weights after slaughter. The same slaughtering procedure was followed in Experiment II and III. In Experiment IV, the hogs were individually-removed and slaughtered as they reached market weight (approximately 180 lb.).

TABLE 1. RATIONS USED

	Experiments I and II		Experiment III[1]				Experiment IV[1]			
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 3	Lot 4	Lot 1	Lot 2	Lot 3	Lot 4
Ingredients	Basal	10% Fat	Basal	10% Fat	10% Fat Plus 7 B-Vit.[2]	Hand Fed Fat, Self Fed Prot.	Basal	10 % Fat Plus 7 B-Vit.[2]	10% Fat Plus 3 B-Vit.[3]	15% Fat Plus 7 B-Vit.[2]
Corn—ground	72.0	62.0	69.5	57.0	57.0	....	69.0	56.0	56.0	50.0
Soybean oil meal—44% protein	12.4	13.3	18.0	19.9	19.9	64.0	18.0	20.2	20.2	21.0
Meat scraps—55% protein	6.1	6.7	6.0	6.6	6.6	20.7	6.0	6.8	6.8	7.0
Alfalfa meal	8.0	8.0	5.0	5.0	5.0	13.8	5.0	5.0	5.0	5.0
Waste beef fat	....	10.0	....	10.0	10.0	*Ad lib.*	....	10.0	10.0	15.0
Ground limestone	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Trace mineralized salt[4]	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53
Bonemeal	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100.53	102.03	100.53	100.53	100.53	100.53	100.03	100.03	100.03	100.03

[1] Aurofac was added to all rations in Experiments III and IV at a level calculated to give 0.5 gm. of aureomycin per cwt. of feed.

[2] B-vitamins were added at the following levels per 100 lb. of feed: Thiamin—1 gm., Riboflavin—230 mg., Niacin—2.33 mg., Pantothenic Acid—1 gm., Pyridoxine—375 mg., Choline—19.4 gm. and Folic Acid—22.7 mg.

[3] Only Thiamin, Riboflavin and Niacin were added at the levels indicated above.

[4] Trace mineralized salt mixture was composed of Iodized Salt—50 lb., Manganese Sulfate—800 gm., Ferrous Sulfate—398 gm., Copper Sulfate—125 gm. and Cobalt Carbonate—10 gm.

TABLE 2. SUMMARY OF RESULTS

Exp.	Lot	No. of Pigs Per Lot	Av. Initial Wt. Lb.	Av. Final Wt. Lb.	Av. No. Days on Test	Av. Daily Gain Lb.	Feed Per Cwt. Gain Lb.	Av. Warm Dressing Percentage[1]	Av. Cold Dressing Percentage[1]	Av. Backfat Thickness[2] Inches	Av. Carcass Grade[3]	Av. Carcass Length[4] Inches	Av. Iodine Number[5]	Manual Carcass Firmness Grade[6]
I	1	3	114	189	60	1.24	436	79.2	77.9	1.64	Ch. No. 1	....	63.5	1.7
	2	4	115	189	60	1.23	408	79.9	78.3	1.54	Ch. No. 1	....	60.7	1.8
II	1	5	121	180	65	0.91	536	79.8	77.5	1.50	Ch. No. 1	....	58.0	2.2
	2	5	122	208	65	1.32[7]	401	78.4	76.4	1.72	Ch. No. 1	....	55.1	2.2
III	1	7	54	191	100	1.37	365	78.3	76.6	1.65	Ch. No. 1	28.0	59.0	1.7
	2	7	54	189	94	1.42	344	77.9	76.1	1.72	Ch. No. 1	27.7	60.1	1.7
	3	7	54	186	86	1.53	332	78.9	76.6	1.70	Ch. No. 1	27.5	....	2.0
	4	7	58	177	97	1.04**	368	73.7**	72.2**	1.31**	Medium	28.3**	....	2.5
IV	1	7	46	185	140	1.00*	395	78.6	78.6	1.46	Ch. No. 1	27.8	....	2.1
	2	7	45	193	106	1.38	355	79.2	79.2	1.55	Ch. No. 1	28.3	....	2.1
	3	7	45	191	117	1.26	371	79.1	79.1	1.59	Ch. No. 1	28.4	....	2.1
	4	7	45	193	109	1.36	346	78.8	78.8	1.56	Ch. No. 1	28.1	....	2.7

* Significant at the 5 percent level.

** Significant at the 1 percent level.

[1] Average dressing percentage = Chilled or hot carcass wt. (head on, leaf fat in) × 100 ÷ final wt. All weights were on chilled carcass except in Experiment IV where hot weights were used.

[2] Average backfat thickness based on measurements at first rib, seventh rib and last lumbar vertebra.

[3] Carcass grades based on U.S.D.A. standards. Grades are Choice No. 1, Choice No. 2, Choice No. 3, Medium and Cull.

[4] Carcass length measured from first rib to aitch bone.

[5] Hanus method used to determine Iodine Numbers.

[6] Grades used were as follows: Hard—1, Medium Hard—2, Medium Soft—3, Soft—4 and Oily—5.

[7] Gilts gained 1.12 and 1.18 lb. per day respectively for Lots 1 and 2, while barrows gained 0.58 and 1.54 lb. respectively.

Carcass Measurements

Thickness of backfat was calculated by averaging measurements taken at the first rib, last rib and last lumbar vertebra. Carcass grades were determined using U.S.D.A. standards for pork carcasses. Carcass firmness was measured manually after chilling for 24 hours at 36° F. Carcass length was measured as the distance from the first rib to the aitch bone. Iodine Numbers were determined on a sample of backfat removed over the 7th rib by the Hanus method as outlined by the A.O.A.C. (1950).

TABLE 3. SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENTS
(Analysis of Variance—Mean Squares)

Exp.	Source of Variation	Degrees of Freedom	Average Daily Gain	Cold Dressing Percentage	Hot Dressing Percentage	Fatback Thickness	Carcass Length	Iodine Numbers	Manual Firmness Grade
I	Total	6							
	Lots	1	0.01	0.8	1.64	0.01		13.	
	Error	5	0.04	2.8	1.87	0.08		67.	
II	Total	9							
	Lots	1	0.44	3.0	5.20	0.12		18.	
	Sex	1	0.02	8.4	5.63	0.01		6.	
	Lots × Sex	1	0.93**	0.1	2.87	0.52*		249.*	
	Error	6	0.07	3.6	2.66	0.06		6.	
III	Total	26							
	Lots	3	0.29*	25.4**	32.83**	0.23**	0.80		
	Error	23	0.03	3.6	3.84	0.04	0.45		
IV	Total	27							
	Lots	3	0.18		0.51	0.02	0.44		0.57
	Error	24	0.06		5.71	0.03	0.86		0.67

* Significant at the 5 percent level.
** Significant at the 1 percent level.

Results and Discussion

Average Daily Gains

In Experiment I, as indicated in tables 2 and 3, the pigs receiving the basal ration (Lot 1) gained approximately the same as those receiving the 10 percent waste beef fat ration (Lot 2). The same can be said for the gilts in Experiment II. The barrows of that experiment showed a difference but the low rate of daily gain for the controls, 0.58 lb., and the fact that the pigs obviously were unthrifty makes it questionable whether the difference should be accepted as significant as suggested by the lot × sex interaction.

In Experiment III, average daily gains of 1.37, 1.42, 1.53 and 1.04 lb. were recorded by Lots 1 through 4, respectively. The difference between Lots 1 (Basal) and 3 (10 percent fat plus 7 B-vitamins)

approached significance. This difference may possibly be attributed to the B-vitamins which were supplied to Lot 3 but not to Lot 1, as Lot 2 (10 percent fat without B-vitamins) gained only slightly more than Lot 1. The lower gains of Lot 4 (self fed protein with fat *ad lib.*), when analyzed statistically, were highly significant at the 1 percent level. This may have been partially due to the fact that the pigs when put on experiment gorged themselves with the fat and went off feed for several days. Then too, the pigs on the self fed protein mixture scoured throughout the feeding period.

In Experiment IV, Lot 1 (Basal) recorded an average daily gain of 1.00 lb., compared to 1.38 lb. for Lot 2 (10 percent fat plus 7 B-vitamins), 1.26 lb. for Lot 3 (10 percent fat plus thiamin, riboflavin and niacin) and 1.36 lb. for Lot 4 (15 percent fat plus 7 B-vitamins). The difference between Lot 1, and Lots 2 or 4 was significant at the 5 percent level and approached significance at the 1 percent level. The difference between Lot 3 and Lot 1 closely approached significance at the 5 percent level.

The addition of waste beef fat to swine rations probably did not result in increases in gains in Experiment I, II or III. Adding of B-complex vitamins to the rations containing fat increased gains slightly, indicating that the vitamin content of the basal ration was either deficient or else the additional vitamins enhanced the effect of the added fat. Satisfactory gains were obtained with mixed rations containing up to 15 percent fat when supplemented with B-vitamins, while very poor results were achieved when the fat was hand fed and a 30 percent protein mixture was self fed.

Feed Per Hundred Lb. Gain

The amount of feed required per hundred lb. of gain for the basal group (Lot 1, Experiments I and II) was 436 and 536 lb., respectively, and for the 10 percent waste fat group (Lot 2) was 408 and 401 lb., respectively (see table 2). In Experiment III, the feed requirements per hundred lb. gain ranged from 332 lb. for Lot 3 (10 percent fat plus thiamin, riboflavin and niacin) to 368 lb. for Lot 4 (hand fed fat plus self fed protein). Lot 4 consumed 67 lb. of fat and 301 lb. of the protein supplement per hundred lb. of gain, eating 18 percent of the total ration in the form of fat. In Experiment IV, the feed requirements were 395 lb. for Lot 1 (Basal), 355 for Lot 2 (10 percent fat plus 7 B-vitamins), 371 for Lot 3 (10 percent fat plus 3 B-vitamins) and 346 lb. of feed for Lot 4 (15 percent fat plus 7 B-vitamins). Differences in feed efficiency were not analyzed statistically, but the lots receiving

waste fat seem to be substantially more efficient in producing gain. This agrees with results by Deuel *et al.* (1947) where addition of 5 to 55 percent fat to the ration increased efficiency of gains in weanling rats.

Backfat Thickness and Carcass Grade

Differences between lots were small and due to variability were not significant, except in the case of Experiment III (tables 2 and 3). In this experiment, the backfat thicknesses were 1.65, 1.72, 1.70 and 1.31 inches, respectively, and a highly significant difference existed between Lot 4 and all other lots. The pigs in Lot 4 gained much more slowly, so the thinner carcasses could be expected.

The average U.S.D.A. swine carcass grade for all groups was Choice No. 1, except for Lot 4, Experiment III, in which the average carcass grade was Medium. Thus, under the conditions of these experiments addition of waste beef fat had little or no effect on backfat thickness.

Carcass Length

Carcass length measurements were taken in only Experiments III and IV. The average lengths for all lots were essentially the same with exception of Lot 4, Experiment III, in which the carcasses were considerably longer than any other lots. This was highly significant when analyzed statistically. On the whole, addition of fat to similar rations did not affect carcass length.

Carcass Firmness

In Experiment I, the average Iodine Number was 63.5 for Lot 1 (Basal) and 60.7 for Lot 2 (10 percent fat). In Experiments II and III, the average Iodine Numbers for Lot 1 were 58.0 and 59.0, respectively, and for Lot 2 were 60.1 and 55.1, respectively. These differences were not significant statistically. The waste beef fat used in these rations was found to have a comparatively low Iodine Number of 43.1. Common feed oils have much higher Iodine absorption values according to Anderson and Mendel (1928). Most workers, including Brown and Morris (1929–30), Ellis and Isbell (1926) and Anderson and Mendel (1928) have shown that the Iodine Number of body fat varies with the Iodine Number of the feed fat. However, the addition of fat to the ration did not affect carcass firmness measurably in this series of trials.

Carcasses were also compared using manual carcass firmness grades. No differences were detected, except for the obviously greater softness of carcasses from Lot 4, Experiment III.

Summary

Four experiments involving a total of 73 pigs were conducted to determine the feeding value of raw ground waste beef fat in swine rations. Levels of 10 and 15 percent fat were fed successfully in the mixed ration, with the replacement value of the fat averaging 115 percent of corn. Added fat increased the efficiency of gains in all experiments. However, there was no consistent increase in average daily gains.

Neither added fat nor B-vitamins showed any clear-cut effect upon dressing percentage, backfat thickness, carcass grades or firmness as measured by Iodine Numbers and manual firmness grades. When the fat was hand fed and the remainder of the ration self fed, the pigs ate 18 percent fat. However, gains were unsatisfactory and the dressing percentage was lower. The carcasses from the pigs which were hand fed fat also had less backfat, more length, and were less firm than those from pigs fed on the other rations.

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THE USE OF MALT SPROUTS IN THE BACON HOG RATION

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IT has been shown by Crampton *et al.* (1953) that choice of feeds can influence the degree of leanness of the bacon carcass. High-energy rations of low fiber and high digestibility and carrying necessary protein levels, when fed to pigs from weaning to about 115 lb., result in rapid growth at the time when muscle is most readily and efficiently produced. If at this point pigs are changed to rations of lower energy value so that even on full feeding they consume less digestible nutrients than otherwise, the excessive fat deposition so undesirable in high grade bacon does not occur.

This "dilution" of energy intake has been accomplished successfully with several feedstuffs, including alfalfa meal, wild oats and wheat bran. Malt sprouts, a barley by-product of the malting industry, carries about 75 percent more crude fiber than wheat bran. Therefore it seemed possible that this feedstuff might replace wheat bran as a diluent of the energy value of bacon hog finishing rations.

Experimental

In Trial 1, malt sprouts was compared with wheat bran as a diluent of the energy value of the finishing ration of bacon hogs. As a result of the findings in Trial 1, malt sprouts was included in the pig ration from weaning to market weight in Trial 2 in order to study the effect of this inclusion on growth during the growing and feeding periods, and on the quality of the carcasses produced. The design of the second trial also provided for a comparison between the properties of malt sprouts and an antibiotic during the growing period.

In both trials, 64 Yorkshire pigs were individually penned and provided with self-feeders. Each pig was weighed every 2 weeks. Feed consumption was determined only at the end of the feeding periods. Upon individually-reaching market weight, animals were slaughtered and carcasses graded and measured by Canadian government graders.

All rations were fortified with a protein-mineral supplement in amounts to supply approximately 16 percent and 14 percent total protein during

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada, Journal Series No. 344.

the growing and finishing periods respectively. This supplement was of the following composition:

	Percent
Meat Meal	25
Fish Meal	15
Linseed Oilmeal	40
Bone Meal	10
Ground Limestone	5
Salt (Iodized)	5

Trial 1

TABLE 1. RATIONS FED

Trial 1

	Ration No.							
Feeds	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)	8 (%)
Pearl barley	70							
Normal barley		90	70	60	50	82	78	74
Wheat bran			20	30	40			
Malt sprouts						8	12	16
Protein-min. suppl.	30	10	10	10	10	10	10	10

Trial 2

	Ration A		Ration B	
Feeds	Growing Period (%)	Finishing Period (%)	Growing Period (%)	Finishing Period (%)
Barley (#1 Feed)	80.0	87.5	72.5	80.0
Malt sprouts	—	—	12.0	12.0
Protein-min. suppl.	20.0	12.5	15.5	8.0

The pigs used in this trial had been fed from weaning to 115 lb. live weight in a previous study in which no differences were found between any of four ration groups. They were allotted at random to eight ration groups for the present test, with the restriction that the 16 pigs of each of the previous experimental lots were distributed two to each of the eight groups of this test. The allotment of animals is indicated in table 2.

TABLE 2. AVERAGE DAILY GAINS, AVERAGE DAILY FEED INTAKES AND CARCASS GRADING RECORDS OF PIGS FED BARLEY AND BARLEY-DILUTED RATIONS

		Ration								
				Normal Barley Plus			Normal Barley Plus			
Sex	Measurement	Pearl Barley 1	Normal Barley 2	20% Bran 3	30% Bran 4	40% Bran 5	8% Malt Sprouts 6	12% Malt Sprouts 7	16% Malt Sprouts 8	All Lots
Male	Gain (lb.)	1.72	1.53	1.57	1.33	1.19	1.94	1.87	1.74	1.61
	Feed (lb.)	6.4	6.2	6.5	6.6	6.4	7.5	7.4	7.1	6.7
Female	Gain (lb.)	1.57	1.83	1.41	1.49	1.18	1.83	2.02	2.01	1.68
	Feed (lb.)	5.7	7.0	6.2	6.6	6.0	7.0	7.3	6.6	6.5
All Animals	Gain[1]	1.66	1.68	1.49	1.41	1.19	1.89	1.96	1.89	1.65
	Feed[2]	5.9	6.6	6.3	6.6	6.3	7.3	7.4	6.8	6.6
	% A Carcasses	100	87.5	75.0	87.5	100	75.0	87.5	75.0	85.0

[1] Necessary Difference between means for statistical significance, 0.32 lb.
[2] Necessary Difference between means for statistical significance, 0.7 lb.

Normal barley comprised the basal feed for all rations excepting that in one lot pearl barley was used because of its low fiber content. For three lots of pigs the normal barley was diluted with 20, 30 and 40 percent of wheat bran. In order to provide comparative rations with corresponding levels of total crude fiber, the normal barley fed to 3 other groups was diluted with 8, 12 and 16 percent malt sprouts.

The composition of the rations is given in table 1.

Trial 2

The general plan of this test was to feed the pigs from a weaning age of about 60 days to final weights of 200 lb., during which time two main rations, A and B, were to be compared. One (A) served as a control and consisted of barley plus the protein-mineral supplement. The second (B) differed from the first only by containing 12 percent of malt sprouts. The composition of these rations is also given in table 1.

The feeding was divided into two periods; the first extended from weaning until the pigs weighed 115 lb. each (growing period), and the second carried them to a market weight of 200 lb. (finishing period). Thirty-two pigs were allotted to Ration A and 32 to Ration B. During the growing period, half the pigs in each of these groups received their rations fortified with 5 gm. of terramycin (TM-5) per ton of feed. The allotment of animals is indicated in table 5.

At the end of the growing period, each of the 4 groups were again divided into 2 sub-groups of 8 animals each, one group in each case being changed either to Ration B or to Ration A, depending on whether it had been on Ration A or B for the growing period. Thus there was opportunity to observe the effect of malt sprouts when fed throughout the market feeding period, when fed only during the finishing period, and when fed only during the growing period. The allotment of animals is indicated in table 6.

Results and Discussion

Trial 1

Live Hog Data. The results of this experiment are, in part, illustrated in table 2. Statistical analysis of the daily gain figures showed no real differences between sexes, and no interaction between sex and kind of ration. There were significant differences between the rations. Removing the hull, bran and germ from the barley did not affect the gain of the pigs. On the other hand, increasing the crude fiber reduced

the gain when wheat bran was the "diluent," but, contrary to expectations, increased it when malt sprouts was employed.

These figures must, of course, also be considered in the light of the feed intake, where there were significant differences between rations. It is evident that the malt sprouts lent extra acceptability to the ration.

When the observed gains were adjusted to the test average of daily feed intake, the relative efficiency of the eight rations showed definite trends. These data are shown in table 3, which includes also the average crude fiber value for the grain portion of each diet, and the net energy as calculated from tables from Morrison's *Feeds and Feeding*.

TABLE 3. RELATION BETWEEN FEED EFFICIENCY AND FIBER AND NET ENERGY CONTENT OF THE RATION

Ration	Percent Crude Fiber	Percent Net Energy	Lb. Gain on Equal Feed[1]
Pearl barley	...	84	1.87
Normal barley	4.9	70	1.71
Barley and 20% bran	6.0	68	1.59
Barley and 30% bran	6.6	67	1.43
Barley and 40% bran	7.2	66	1.33
Barley and 8% malt sprouts	6.1	69	1.69
Barley and 12% malt sprouts	6.7	69	1.73
Barley and 16% malt sprouts	7.3	68	1.83

[1] Necessary difference between mean values for statistical significance, 0.22 lb.

The higher efficiency of the practically fiber-free pearl barley ration was in accordance with expectations. The gradual reduction in feed efficiency as normal barley was diluted with progressively greater amounts of wheat bran was also predicted. However, the complete reversal of ration efficiency resulting from the introduction and progressive increase in malt sprouts was not at all anticipated. In these rations, the fiber content paralleled closely that of the wheat bran lots. Since increasing the malt sprouts increased both gains and gains adjusted to equal feed intake, it must be concluded that some factor in this feed, obviously other than crude fiber, was responsible. Its direct effect was seen in the increase in voluntary feed intake which was of the order of 12 percent over the comparable bran-fed lots.

Statistically, not all of the differences between the eight lots were significant. However, the trends for bran in one direction, and for malt sprouts in the other, were unmistakable.

Carcass Data. Over the whole test there were 85 percent grade A carcasses which was higher than usual. Nevertheless, it is evident that the

inclusion of malt sprouts did not affect the grading much over that found on the barley alone. There was a trend to higher grades with increasing bran dilution, but since the same fiber intake from malt sprouts was accompanied by increased rather than decreased gains, it was not surprising that the leanness of carcass was not increased in the lots fed malt sprouts.

Trial 2

Replicate 1. The original plan of this experiment called for the supplementation of half the pigs on Rations A and B during the growing period with 2 gm. of procaine penicillin plus 3 mg. of vitamin B_{12} per ton of feed. However, shortly after the trial was started, scouring developed in a considerable number of the pigs. The condition increased

TABLE 4. INCIDENCE OF SCOURS IN PIGS (16 ANIMALS PER LOT)

Supplementation	Ration A (control)	Ration B (12% malt sprouts)
None	69%	25%
Penicillin and vitamin B_{12}	81%	44%

rapidly in severity until more than half the pigs were affected. At this time the test was discontinued, the animals disposed of, and the housing quarters disinfected. Salvaged from this test was some information on the effect of the different rations on the incidence of scours. These data are shown in table 4.

It is of interest that, regardless of whether or not the control ration was supplemented with penicillin and vitamin B_{12}, the addition of malt sprouts apparently decreased the incidence of scours. It is recognized that the levels of supplementation with penicillin and vitamin B_{12} were below the recommended amounts, and there is the possibility that increased intake of penicillin might have had a protective influence against scouring comparable to that exhibited by malt sprouts.

In any case, the objectives of the test as designed were not realized because of this epidemic. Therefore, a second experiment was organized which was a replicate in all respects of the first, except that 5 gm. of terramycin per ton of feed replaced the penicillin and vitamin B_{12} used in the first test.

Replicate 2. For various causes not of nutritional origin, 4 pigs from the control ration of the growing period, and an additional 6, one from

each of six of the eight ration groups of the finishing period, failed to complete the test. Any data relevant to these animals are deleted in calculating the averages.

For the growing period, the average daily gain and feed consumption, as well as feed efficiency, as illustrated by the average daily gain adjusted to the average feed intake of the test, are given in table 5.

It is evident from the figures in table 5 that the addition of 12 percent malt sprouts to the barley ration increased the rate of gain of the pigs significantly, and improved the efficiency of the ration. When either

TABLE 5. AVERAGE DAILY GAIN AND FEED CONSUMPTION, AND THE GAINS ADJUSTED TO CONSTANT FEED INTAKES—GROWING PERIOD

Antibiotic Supplementation	Measurement (lb.)	Ration A (control)	Ration B (12% malt sprouts)
		(12 pigs)	(16 pigs)
None	Daily gain*	1.04	1.24
	Daily feed consumption	3.45	3.98
	Adjusted gain	1.09	1.25
		(16 pigs)	(16 pigs)
5 gm. terramycin per ton of feed	Daily gain*	1.41	1.38
	Daily feed consumption	4.32	4.44
	Adjusted gain	1.39	1.35

* Differences greater than 0.10 lb. between gain per day, 0.24 lb. between feed consumption, or 0.09 lb. between adjusted gains are statistically significant at P of 0.05.

the barley or barley plus malt sprouts ration was supplemented with terramycin, the rates of gain and feed efficiencies were not differentially altered. However, they were both significantly higher than the corresponding measurements for the unsupplemented barley plus malt sprouts ration.

This information indicates that malt sprouts is capable of stimulating the growth of pigs from weaning to 115 lb., but that its growth stimulating properties are inferior to those of terramycin.

The results for the finishing period are found in table 6. The figures are arranged to show the rations which the respective pigs received during the growing period.

For the pigs which during the growing period served as controls, having neither terramycin nor malt sprouts, the introduction of malt sprouts into the ration at the beginning of the finishing period resulted in no growth stimulation. In fact, the gains made by these two comparable groups of pigs, as well as their feed efficiencies, were prac-

tically equal. This is in contrast to the findings in Trial 1. These opposing results are difficult to reconcile, and serve mainly to point out the necessity of further experimentation with respect to the growth

TABLE 6. AVERAGE DAILY GAIN AND FEED CONSUMPTION, FEED EFFICIENCY AND PERCENTAGE OF GRADE A HOGS—FINISHING PERIOD

Rations Fed During Growing Period		Measurement		Finishing Period Rations* Ration A (control)	Ration B (12% malt sprouts)
				(5 pigs)	(6 pigs)
No antibiotic	Ration A (control)	Daily gain	(lb.)	1.53	1.53
		Daily feed cons.	(lb.)	6.62	6.26
		Adjusted gain	(lb.)	1.43	1.50
		Grade A carcasses	(%)	100	80
				(8 pigs)	(7 pigs)
	Ration B (12% malt sprouts)	Daily gain	(lb.)	1.27	1.55
		Daily feed cons.	(lb.)	5.51	6.71
		Adjusted gain	(lb.)	1.40	1.43
		Grade A carcasses	(%)	86	86
				(6 pigs)	(8 pigs)
5 gm. terramycin per ton feed	Ration A (control)	Daily gain	(lb.)	1.42	1.52
		Daily feed cons.	(lb.)	5.88	6.08
		Adjusted gain	(lb.)	1.47	1.53
		Grade A carcasses	(%)	80	88
				(7 pigs)	(7 pigs)
	Ration B (12% malt sprouts)	Daily gain	(lb.)	1.50	1.48
		Daily feed cons.	(lb.)	6.28	6.00
		Adjusted gain	(lb.)	1.47	1.51
		Grade A carcasses	(%)	71	85

* Differences greater than 0.20 lb. between gain per day, 0.75 lb. between feed consumption, or 0.13 lb. between adjusted gains are statistically significant at P of 0.05.

stimulating properties of malt sprouts during this period of growth in the pig.

With respect to the pigs which during their growing period received terramycin but no malt sprouts, the introduction of malt sprouts at the beginning of the finishing period resulted in a statistically insignificant increase in gain and feed efficiency. Of those that received both supplements during the growing period, the removal of malt sprouts at the

conclusion of this period was without effect as compared to its continuance. Whether this represented a sufficient storing of antibiotic from the growing period so that no drop in growth was experienced cannot be ascertained from this trial.

However, for those pigs which during the growing period had received the malt sprouts but no antibiotic, the removal of the malt sprouts at the beginning of the finishing period caused a decline in the rate of gain as compared to those that had the sprouts continued in the ration. This effect is one which is usually noted when an antibiotic supplement is removed from the ration at this stage in the feeding period (Hanson *et al.*, 1952; Wallace *et al.*, 1953). It should also be noted that the chief effect of the removal of malt sprouts from the ration was a reduction in feed intake. This, again, is a response characteristic of the removal from the ration of an antibiotic supplement at this stage of feeding (Hanson *et al.*, 1952; Wallace *et al.*, 1953).[2]

Table 6 also shows the carcass grades of the pigs fed in this trial. The gradings do not show any consistent trend which can be traced to ration. Only 8 carcasses were degraded for excessive fat, and these were distributed through all but one of the ration groups. The data indicate that malt sprouts did not affect carcass quality.

Summary

The results of these feeding trials indicated a remarkable similarity between the effects of malt sprouts and antibiotic supplements when added to the hog ration.

Malt sprouts appeared to be partially effective in preventing at least one type of scours.

The inclusion of malt sprouts in the growing ration of the pig resulted in a stimulation of growth and a significant increase in feed efficiency. The growth stimulation was not as great as that obtained with terramycin.

When malt sprouts was included in the hog finishing ration, a growth stimulating effect was observed for one lot of pigs. The feed efficiency of the ration also showed an upward trend as the malt sprouts fraction of the ration was increased. However, the introduction of malt sprouts

[2] These similarities prompted the examination of malt sprouts for antibiotical substances, but bacteriological studies carried out by Professor R. H. Wallace of the Bacteriology Department of Macdonald College, revealed negative results. However, malt sprouts was found to contain one or more unidentified growth factors which are soluble in water and heat stable. The test organisms used were (1) *Bacillus mesentericus* (2) *Salmonella enteritidis* (3) *Eberthella typhosa* (4) *Bacterium coli* (5) *Klebsiella pneumoniae* (6) *Proteus vulgaris* (7) *Pseudomonas aeruginosa* (8) *Staphylococcus aureus and* (9) *Salmonella paratyphi.*

into the finishing ration neither stimulated growth nor increased feed efficiency for a second lot of pigs. These opposing results cannot be reconciled, and point to the necessity of further research into the growth-stimulating properties of malt sprouts during this period of growth in the pig.

The withdrawal of malt sprouts from the ration when pigs reached a weight of 115 lb., resulted in a decrease in growth rate, presumably due to the decline in feed intake. Such a decrease in growth rate was not observed with the withdrawal of malt sprouts from a ration previously supplemented with terramycin.

The crude fiber introduced into the ration by the use of malt sprouts apparently in no way decreased the value of the ration for pigs. It was not, however, effective in improving the grade of carcass produced.

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INHIBITION OF HEAT BY PROGESTERONE AND ITS EFFECT ON SUBSEQUENT FERTILITY IN GILTS [1, 2]

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APPROPRIATE doses of progesterone have been shown to inhibit heat and ovulation in both sheep (Dutt and Casida, 1948 and O'Mary *et al.*, 1950) and cattle (Christian *et al.*, 1948, Willett, 1950 and Ulberg *et al.*, 1951); satisfactory fertility also has been obtained on a limited number of animals at the heat subsequent to the inhibitory treatment. Ulberg *et al.* (1951) found in swine that a suitable dose of progesterone would inhibit heat and ovulation; however, the subsequent normal-appearing estrous periods were not tested for fertility. Further, they found that sub-optimal doses gave aberrant estrual behavior and resulted in variable numbers of cystic follicles and corpora lutea. This experimentally-producable ovarian state appears similar to spontaneous cases of "incomplete" ovulation—i.e., presence of both corpora lutea and cystic follicles (Warnick *et al.*, 1949, Wilson *et al.*, 1949 and Wiggins *et al.*, 1950). Further investigation appears justified to develop a dependable method of synchronizing heat periods of different animals which might have some use in swine breeding and also to determine the effect of incomplete ovulation on the potential fertility of the animal.

General Procedures

Gilts were allotted randomly to the control and experimental groups. Heat checks were made twice daily; the first day of heat was designated as day-1 and all day-measurements were made from it. Treatments were begun on either day-10 or -15 and consisted of daily injections through day-28. All gilts were bred at the first heat following the beginning of the injections. Crystalline progesterone in corn oil at a concentration of 25 mg. per cc. was injected into the backfat or muscle of the upper-shoulder area of the experimental animals. Corn oil alone was injected into the controls. Within each group, one-half was slaughtered one to

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two days after the end of heat (3-day gilts); while the remaining one-half was slaughtered on the 25th day after the onset of heat (25-day gilts). When a gilt came into heat during treatment, the treatment was stopped, the gilt bred and then slaughtered in accordance with the previously determined plan. If an experimental gilt did not exhibit heat by the eighth day following cessation of treatment, she was slaughtered; if a gilt showed persistent estrus, she was slaughtered at the end of 5 days of heat. The data collected at the time of slaughter were (1) number of follicles 10 mm. or more in diameter (follicular cysts), (2) number of corpora lutea (number of ovulations), (3) number of fertilized and unfertilized ova recovered in the 3-day gilts, and (4) number of normal and abnormal embryos in the 25-day gilts. The number of lost ova was estimated by subtracting the number of recovered ova from the number of corpora lutea. This estimate includes ova entrapped in the follicle, lost in the body cavity and unrecovered because of faulty technique. The proportions of the different kinds of lost ova are not known, but it is believed that the last named kind is of only minor importance.

Trial I—Comparison of High and Low Doses

The first trial was designed to test two levels of progesterone: high—to give an expected normal response, and low—to give an expected abnormal response, i.e., incomplete ovulation.

Twenty-nine gilts of mixed breeding (approximately equal numbers purchased from two different herds) were divided into three groups. Treatments were as follows: (1) high dose—100 mg. progesterone in oil daily, (2) low dose—25 mg. progesterone in oil daily, and (3) control—4 cc. of corn oil daily. All injections were begun on day-15 of the cycle. This experiment was started August 31 and ended December 2, 1952. Three gilts were discarded at autopsy—one from the controls (because of bursitis and pyosalpinx) and one each from the experimental groups (one had pyometra, the other an incomplete uterine horn).

Low Dose

Only one of the 4 gilts in the 3-day group subjected to the low-level of progesterone (table 1) came into heat and she showed persistent estrus; none of the 5 gilts in the 25-day group showed estrus. Seven out of the total of 9 gilts had cystic ovaries (follicles 10 mm. or more in diameter) with no corpora lutea when slaughtered. The two remaining gilts had shown typical signs of approaching estrus (swollen vulva,

TABLE 1. EFFECT OF PROGESTERONE ADMINISTRATION ON THE SUBSEQUENT FERTILITY OF GILTS

Comparison	Trial I			Trial II		Trial III			Combined Data	
	Control	100 mg. per Day	25 mg. per Day	Control	100 mg. per Day	Control	100 mg. per Day Beginning Day-15	100 mg. per Day Beginning Day-10	Control	100 mg. per Day Beginning Day-15
No. showing heat, all gilts	8 (8)[1]	9 (9)	1 (9)	14 (14)	13 (15)	11 (11)	12 (12)	9 (10)	33 (33)	34 (36)
Av. lgth. heat (days), all gilts	2.6 (8)	2.1** (9)	5.0 (1)	2.6 (14)	2.5 (15)	2.1 (11)	2.0 (12)	2.1 (10)	2.5 (33)	2.2* (36)
Av. interval[2] (days), all gilts	0.0 (8)	5.6 (9)	6.0 (1)	0.0 (14)	5.3 (13)	0.0 (11)	6.7 (12)	5.9 (9)	0.0 (33)	5.8 (34)
No. showing foll. cysts, 3-day	1 (5)	0 (4)	4 (4)	2 (6)	5 (8)	0 (5)	4 (6)	1 (4)	3 (16)	9 (18)
Av. no. foll. cysts, 3-day	0.2 (5)	0.0 (4)	21.2 (4)	0.7 (6)	8.0 (8)	0.0 (5)	9.7* (6)	4.0 (4)	0.3 (16)	6.8* (18)
No. showing corpora lutea, 3-day	5 (5)	4 (4)	0 (4)	6 (6)	6 (8)	5 (5)	4 (6)	4 (4)	16 (16)	14 (18)
Av. no. corpora lutea, 3-day	16.0 (5)	16.8 (4)	0.0 (4)	12.7 (6)	12.1 (8)	12.2 (5)	11.0 (6)	14.2 (4)	13.6 (16)	12.8 (18)
Av. no. ova lost,[3] 3-day	1.6 (5)	2.0 (4)	0.0 (4)	3.0 (6)	6.5 (8)	0.8 (5)	4.0 (6)	2.2 (4)	1.9 (16)	4.7 (18)
Av. no. unfert. recovered ova, 3-day	1.4 (5)	5.5 (4)	0.0 (4)	0.5 (6)	0.9 (8)	0.4 (5)	3.3 (6)	7.8[4] (4)	0.8 (16)	2.7 (18)
Av. no. fert. recovered ova, 3-day	13.0 (5)	9.2 (4)	0.0 (4)	9.2 (6)	4.8 (8)	11.0 (5)	3.7* (6)	4.2 (4)	10.9 (16)	5.4* (18)
Av. no. normal embryos, 25-day	13.7 (3)	4.6* (5)	0.0 (5)	5.4 (8)	1.3 (7)	6.8 (6)	4.7 (6)	9.8 (6)	7.4 (17)	3.3 (18)

* Probability of 0.05.
** Probability of 0.01.
[1] Number in parenthesis indicates number of gilts observed and the numbers on which averages are based.
[2] Number of days between end of injections and onset of heat.
[3] Number of corpora lutea minus number of recovered ova.
[4] Probability of 0.08.

riding other gilts, and interest in the boar) on about day-21 of the estrous cycle during treatment but would not allow the boar to mount. Upon slaughter, their ovaries were found to contain corpora lutea (16 in each gilt) appearing old enough to have resulted from ovulation during the partial heat. Thus, these low-dose gilts responded in a manner in part similar to each of two groups reported by Ulberg *et al.* (1951): 25 mg. group—ovulation during treatment, and 50 mg. group —high incidence of cystic ovaries without corpora lutea.

High Dose

The high-dose gilts developed no cystic follicles and reacted similar to the 100 mg. group of Ulberg *et al.* (1951). All of them exhibited heat subsequent to treatment, but the average length of heat for them was shorter than for the controls (2.1 as compared to 2.6 days).

The factors most responsible for the marked reduction in the number of embryos at 25 days in the 100 mg. experimental group were—failure of fertilization (5.5 unfertilized ova as compared to 1.4 in the controls), and embryonic death (estimated loss in the experimental group, 4.6 embryos (9.2 minus 4.6) and in the control, 0 embryos (13.0 minus 13.7), the apparent negative value presumably being due to sampling error).

Trial II—Effect of High Dose on Fertility

The high-dose group in trial I gave strong indication of some interference with fertility. Trial II was conducted to test for such results specifically.

Thirty-one crossbred Chester White-Poland China gilts were divided into two groups: control—receiving corn oil only, and experimental—receiving 100 mg. progesterone in oil daily. All injections were begun on day-15 of the estrous cycle. This experiment was started on January 6 and ended March 25, 1953. Two gilts were discarded—one from the control group because she showed a slight bursitis and salpingitis at autopsy and one from the experimental group because of injury at the time of mating.

The response of these gilts to the high-dose lacked agreement with that of their predecessors in one important aspect—the progesterone treatment increased the number of follicular cysts greatly (8.0 as compared to 0.7 for the controls, table 1); whereas in the previous trial and in the work reported by Ulberg *et al.* (1951), no increase had been observed following treatment with the 100 mg. dose. The increased

number was contributed mainly by 3 gilts which themselves averaged 19. The markedly smaller number of normal embryos at 25 days in the experimental group (1.3 as compared to 5.4 in the controls) resulted primarily from more lost ova (6.5 as compared to 3.0).

Trial III—Comparison of Injections Begun on Days 10 and 15

The appearance of follicular cysts in the progesterone treated gilts of trial II, prompted further investigation of their possible cause. It had been observed (Ulberg *et al.*, 1951) that the starting-time of injections in the cycle might affect the character of the results—i.e., injections started on day-19 inhibited heat less consistently than those started on day-15. One of the principle differences between trial I and II was the source and breeding of the gilts. Day-15 in our sample of gilts may have been approximately at the border-line with respect to the time of regression of the corpora lutea—i.e., injections started on day-15 found some gilts with yet fully functional corpora lutea and supplementation with progesterone on that day prevented follicular development, as occurred in trial I; while in other gilts, the function of the corpora lutea had declined to such an extent before day-15 that administration of exogenous progesterone did not inhibit follicular growth but did inhibit ovulation with a cystic condition resulting, as in trial II. One physiological difference between the gilts of trial I and II was noted: the numbers of corpora lutea in the controls of the two trials were 16.0 and 12.7, respectively. Whether or not the lower ovulation rate in the gilts of the second trial is associated with a decline of ovarian function earlier in the estrous cycle is of course not known. A second difference between trials I and II was that injections were given by different individuals in the two trials. Depth of injection may have varied and the kind of tissues in which the hormone was deposited may possibly have affected the rate of mobilization of the hormone. However, variation in physiological stage at time of injection was thought to be a more likely explanation, and therefore, this trial was designed to test the theory by starting injections earlier in the cycle in one of the experimental groups.

Thirty-five gilts of mixed breeding were purchased from a local stockyard and divided randomly into three groups and treated as follows: (1) early dose—19 daily injections of 100 mg. progesterone in oil beginning on day-10, (2) late dose—14 daily injections of 100 mg. progesterone in oil beginning on day-15, and (3) control—daily injections of corn oil beginning on day-10. This trial was started on May

20 and ended August 3, 1953. Two gilts were discarded because of injury incurred at the time of mating.

Early-Injected

The performance of the early-injected group is more similar to that of the control group than is that of the late-injected group with the possible exception that the average number of unfertilized ova in the early-injected group exceeded either of the other two groups (table 1). However, this occurrence is inconsistent with the fact that the average number of normal embryos for the group exceeded the controls by 3. Sampling error in one or both of these observations is presumed to account for the discrepancy.

Late-Injected

The response of the late-injected gilts was similar to that of the correspondingly treated gilts in trial II (table 1). The uniformity with which the cystic condition appeared in the gilts of the two trials when subjected to the high-dose beginning on day-15 should be noted. Five out of 8 and 4 out of 6 gilts (trials II and III, respectively) were "cystic" to varying degrees; and the over-all average numbers of cysts for the two groups were 8.0 and 9.7.

In trial III, the number of follicular cysts in the late-injected group was greater than in the control gilts (9.7 as compared to 0.0). The smaller number of fertilized ova (3.7 as compared to 11.0) is an indication of the combined influence of the slightly lower number of corpora lutea, greater ova loss and higher fertilization failure.

Trials I–III—Combined Data

Data from the three trials were combined (table 1) for evaluation of the over-all effects of the 100 mg. dose of progesterone started on day-15. It was suspected that the distributions of some of the variables under consideration were not normal. A ranking method for examining the data, the H test of Kruskal and Wallis (1952), therefore was used. The H values (which are close approximations of Chi-square values) for each trial were pooled for an over-all test of significance (Fisher, 1944). The pooling of these values of course assumes that significant differences in treatment effect do not exist between trials. Inspection of the data leads one to believe this is true except in the case of the number of follicular cysts in trial I.

Three phenomena were affected significantly by the treatment: the length of estrus subsequent to the progesterone treatment was shortened (2.2 as compared to 2.5 days); the production of cysts was increased (6.8 vs. 0.3) but virtually all of it in trials II and III; and the number of fertilized ova was lowered (experimental 5.4, control 10.9). This marked difference (5.5 ova) was brought about as a cumulative result of the effect of progesterone on three antecedent characters: (1) number of ovulations depressed (12.8 as compared to 13.6), (2) number of lost ova increased (4.7 vs. 1.9) and (3) fertilization failure increased (2.7 as compared to 0.8 unfertilized ova for controls).

The small number of normal embryos in the experimental group at 25 days (3.3 as compared to 7.4 for controls) reflects the differences in

TABLE 2. EFFECT OF 100 MG. OF PROGESTERONE AND PRESENCE OF OVARIAN CYSTS[2] ON THE REPRODUCTIVE BEHAVIOR OF GILTS WHICH OVULATED

Comparison	Progesterone Treated		Control	
	Cystic	Non-Cystic	Cystic	Non-Cystic
Av. no. Corpora lutea	18.5 (4)[1]	16.1 (9)	15.3 (3)	13.2 (13)
Av. no. cysts	1.8 "	0 "	1.7 "	0 "
Av. no. lost ova	11.0 "	3.2 "	3.0 "	1.6 "
Av. no. unfert. ova	2.5 "	4.3 "	0.3 "	0.8 "
Av. no. fert. ova	5.0 "	8.6 "	12.0 "	10.7 "

[1] Number of observations.
[2] Limited to 1 to 3 cysts per gilt.

the antecedents discussed above and presumably also reflects any possible differences in embryonic death. Embryonic death is represented in this discussion as a percentage of the recovered fertilized ova; it is the quantity (average number of recovered fertilized ova minus the average number of normal embryos at 25 days) stated as a percentage of the average number of recovered fertilized ova. The embryonic death for the experimental group was 39 percent and for the control 32 percent. Thus, over all trials, the proportion of fertilized eggs resulting in dead embryos was not appreciably greater in the progesterone treated gilts than in the controls.

Cysts were found in the ovaries of both the control and the experimental animals. To investigate the effects of cysts as well as progesterone on fertility, the data were studied in the following manner. Only gilts having corpora lutea were considered (table 2). The control groups

and the experimental groups (high-dose, late-injected) were each divided into two classes—with and without cysts. The maximum number of cysts in any one gilt of the control group was 3. Therefore, one gilt with more than 3 cysts in the experimental group was eliminated, thereby equalizing the two groups approximately with regard to the average number of cysts and making this factor independent of the progesterone treatment.

The number of corpora lutea was significantly greater in the experimental than in the control group (16.8 and 13.6, respectively).

A significant interaction existed with regard to the number of lost ova between experimental vs. control and cystic vs. non-cystic. The data indicate that the number of lost ova was more markedly increased by the cystic condition in the experimental group (11.0 vs. 3.2 ova) than in the control group (3.0 vs. 1.6 ova). The endocrine activity of those cysts appearing in the progesterone-treated animals may not be the same as the activity of those cysts in the controls.

The average number of unfertilized ova was significantly greater in the progesterone treated group than in the control group (3.8 and 0.8, respectively); while statistically, cysts were not a factor in fertilization failure (average 1.6 where they were present and 2.3 where they were not).

Discussion

Ulberg *et al.* (1951) have suggested that higher dosages of progesterone inhibit heat and ovulation by blocking the gonadotrophic complex and that lower dosages are more effective against the luteinizing action than against the follicle stimulating, thereby accounting for the production of cysts. Our data suggest no change in the above theory, but the appearance of cysts in the gilts on the high-dose in two out of three of our experiments indicates that further work is needed to determine the factors causing the variation. The fact that initiation of injections on day-10 in trial III produced fewer cysts than injections beginning on day-15 is being interpreted that stage of cycle at initiation of treatment will affect the results, and that probably individual variation in level of corpus luteum function on a critical day near the end of its function (such as day-15) will also cause variation. Differences between whole groups at this critcal stage could be visualized readily as having either a genetic or an environmental base.

The greater ovulation rate in the experimental gilts (when consider-

ing only those which ovulated) may have been the result of the extended estrous cycle, i.e., the additional time between heats allowing more follicles to develop to a gonadotrophin-sensitive stage.

A latent effect of progesterone may have been responsible for the shorter estrous periods and for the increase in fertilization failure. For the latter, it is suggested that progesterone has a persisting inhibition on the uterine and oviduct muscular movements interfering with sperm transport. The larger ova loss particularly in the progesterone-treated, cystic animals may have resulted from a persisting interference of progesterone (complicated by the endocrine activity of the cysts) with the muscular activity of the infundibulum allowing more ova to be lost in the body cavity.

Summary

Ninety-five gilts of mixed breeding were used in three trials to test their response to injections of crystalline progesterone (levels of 25 and 100 mg. daily started on day-10 or -15 of the estrous cycle and continued through day-28) with the objectives being (1) to develop a treatment which would inhibit heat and ovulation and would be followed by a fertile heat, and (2) to observe the effects of incomplete ovulation on fertility.

Heat was inhibited by progesterone on both dosages and at both times of onset of injections. However, the fertility of the heats following these injections was *nil* in those gilts on the 25 mg. dose and low for those on the 100 mg. level with injections begun on day-15.

The latter showed the following results: Heat was inhibited in all gilts during treatment. The heat period subsequent to treatment (shown by 34 of 36 gilts) was shortened by an average of 0.3 days. The average number of cysts in the 18, 3-day gilts, observed following treatment, was increased by 6.5 over the controls. Four of these 18 gilts had only cysts. The average number of fertilized ova was decreased by 5.5 ova (reflecting ovulation decrease, ova loss and fertilization failure). Embryonic death was not materially altered by the progesterone treatment.

Those control and experimental gilts which had ovulated were divided into cystic and non-cystic subgroups. It was then found that progesterone increased the ovulation rate over all experimentals by 3.2 ova. There was a much greater increase in number of lost ova in the cystic gilts over the non-cystics, both receiving progesterone, than in similarly

classed gilts not receiving progesterone. Fertilization failure was significantly increased over all progesterone groups but was not affected by the cystic condition.

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AN IMPROVED METHOD FOR DETERMINING BOMB CALORIMETER VALUES

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THE method presented herein utilizes a peroxide bomb calorimeter [2] and a recording potentiometer equipped with a maganin-nickel thermocouple. The advantages afforded by the recording potentiometer are, first, the temperature change is easily determined and second, a permanent record of the determinations is made.

The bomb is ignited with an electric ignition unit [3] and is rotated by an electric motor [4] driving through an elastic band (figure 1).

The recording potentiometer used in this laboratory is a Brown Electronic Strip Chart Recorder and Indicating Potentiometer.[5] The scale is graduated from 18° C. to 28° C. and readings can be estimated with good accuracy to the nearest hundredth of a degree. The 6″ steel resistance thermometer is connected to the potentiometer with a 36″ conductor cable, both of which are supplied by the instrument manufacturers. Our recorder operates at a speed of 10 inches per hour and is equipped with an operational pen.

While directions are provided with the calorimeter, the following comments may be helpful to some. When ignited, the bomb contains 1 gm. of potassium perchlorate, about 15 gm. of sodium peroxide, plus the sample, all thoroughly mixed together. One-half gram of sample is usually used but for samples containing a high percentage of fat (20 percent or more) we reduce the quantity to one-quarter of a gram. In addition it has been found that the inclusion of 0.2 grams of standard combustion mixture (see footnote 3) facilitates ignition with all samples.

It is usually possible to make three determinations over the 10 degree range provided by the potentiometer before it is necessary to change the water in the calorimeter. The initial temperature is read from the chart as the starting point of the horizontal curve. The final temperature is read at the point where the curve changes to a straight line. This can be determined easily with the help of a ruler or other straight edge.

Experience in our laboratory indicates that temperature changes

[1] Contribution from the Faculty of Agriculture, McGill University, MacDonald College, Que., Canada. Journal Series No. 336.

[2] Parr Instrument Company, Moline, Ill. Model No. 1401.

[3] Parr Instrument Company, Moline, Ill.

[4] Cenco Variable Speed Stirrer set at slow speed.

[5] Minneapolis-Honeywell Regulator Co. Model No. 153X11W-X-28P4, using Chart No. 5947-1.

due to radiation are insignificant and may be disregarded. The *observed* temperature rise is corrected for fuse, accelerator, hydration, etc., by subtracting the value noted on the label of the bottle of standard combustion mixture. The *corrected* temperature rise is multiplied by the water equivalent of the bomb; adjusted for the combustion mixture

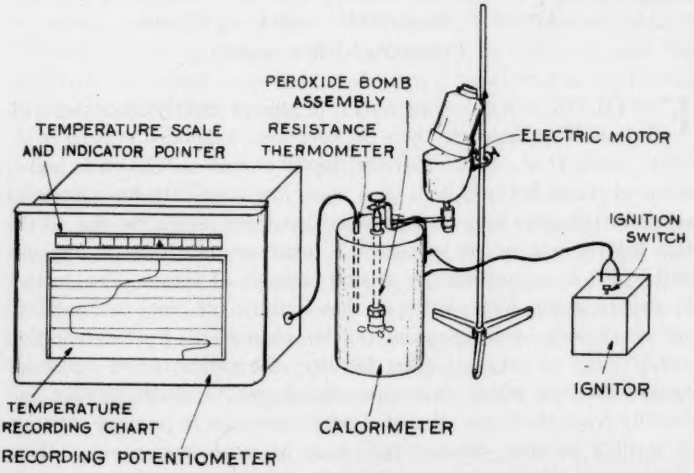

Figure 1. An Improved Method for Determining Bomb Calorimeter Values.

present and amount of sample used; and the final answer expressed as Calories per gram. A sample calculation is shown below:

Observed temperature rise, °C.	2.43
Correction for fuse, accelerator, etc., as on label of combustion mixture °C.	0.20
Corrected temperature rise °C.	2.23
Multiply by 1439 Cals., water equivalent of the bomb	3210 Cals.
Correction for combustion mixture (—1334 Cals.)	1876 Cals.
Correction for sample size (for 0.5 gm. = x 2)	3752 Cals.
K cals. per gram sample	3.75 Cals.

An analysis of variance was carried out on about 1000 determinations on feed and feces samples. The values determined when using the recording potentiometer with 95 samples showed a standard deviation which was 3.0 percent of the mean. In a comparable set of values determined on some 900 samples, with the temperature rise read from the usual type thermometer, the standard deviation was 3.4 percent of the mean.

THE INDEPENDENCE OF MOTILITY AND FERTILITY AS PHYSIOLOGICAL PHENOMENA OF RABBIT SPERMATOZOA [1]

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FERTILITY of bull spermatozoa is in general slightly correlated with their motility; however, there has been some indication (Cheng *et al.*, 1949; Stone *et al.*, 1950) that the degree of this association is higher when measured between bulls than when measured between samples of semen of the same bull. It is possible that these results are due to the fact that no one sample of semen is tested as reliably as is any one bull which is represented by several samples of semen. The further hypothesis is put forth, however, that fertility (*in vivo*) and motility (*in vitro*) may be independent cellular phenomena. Factors affecting motility may or may not affect fertility; the nature of the particular factors that are acting determines the degree to which fertility and motility respectively are affected. Factors operative to produce variation in motility between different bulls may be more constant than those that cause variation between different samples from the same bull. Cheng and Casida (1948) demonstrated a significant association between motility and fertility of rabbit spermatozoa when motility was modified by the use of high dilutions of Kreb's solution. That work suggested the experimental animal for as well as the particular experimental approach to the analysis of the relation of motility and fertility used in this study. The sacrifice of a relatively large number of animals was necessary and this could not be done practically with cattle. Although the results obtained with rabbits are not necessarily true for cattle and other farm animals, yet there seems little reason to assume that the fundamental relationships between motility of spermatozoa and their fertilization capacity differs greatly among the higher mammals.

Spermatozoa were subjected to different factors known to affect their motility and the estimates of concomitant fertility were then tested for differences between factors. Motility and fertility variations due to pH and osmotic pressure differences in the sperm diluent have been studied.

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[2] On study leave from Faculty of Agriculture, Ibrahim University, Egypt.

Diluters using four different buffer systems (mono and disodium phosphate, mono and dipotassium phosphate, sodium citrate and citric acid) were used. The first study was designed to determine the optimum pH for the support of motility for each "salt" and also a different pH which would support some motility but at low level. The optimum molality for motility was also determined as well as a hypertonic and hypotonic concentration which would support a low level of motility. These particular variations in pH and molality for each salt were then studied in the second experiment to determine their effect on the relation between motility and fertility.

Experiment 1

Material and Methods

Ten sexually mature male rabbits of known fertility and on a regular semen collection schedule were used. Semen was collected from these bucks at intervals of two to three days by the artificial vagina (Macirone & Walton, 1938). Collections were made from five or more males for each trial of the experiment. The semen was mixed well and the motility of the spermatozoa checked microscopically to avoid use of any composites of markedly low motility. None, however, was discarded for this reason.

Diluents of different molality and pH were prepared for each of the four different salts (15 for each or a total of 60). The salts used will be referred to as sodium phosphate, sodium citrate, potassium phosphate, and potassium citrate. It was of interest to compare the effects of two different positive ions in the presence of the same negative ion or two different negative ions in the presence of the same positive ion. Molalities of .04, .07, .10, .13 and .16 were used for each salt and three different pH levels (6.5, 7.0 and 7.5) were tested at each of these molalities.

A mixture of the alkaline salt and the acid salt in the case of phosphates or the alkaline salt and the acid in the case of citrates, both of the same molality, was made to get the required pH level as determined by the Beckman pH meter. The different diluents used in the experiment are indicated in the skeleton of table 1.

Semen collected as indicated above (0.1 ml.) was diluted with each of these diluents. One part of semen was used to nine parts of diluent. The different diluents at room temperature were added drop by drop to the semen in 2 ml. vials and covered with a layer of pure paraffin oil and stoppered. The vials were then wrapped in cotton and stored in a thick walled paper-board box in the refrigerator at approximately 5° C.

for six hours. The wrapping of the vials in the described manner was intended to avoid temperature shock of the spermatozoa. A preliminary study indicated that approximately 45 minutes were required for the spermatozoa so treated to be cooled to refrigeration temperature. This storage period was chosen arbitrarily as one which might be expected to be long enough for differences between the diluents in the motility of

TABLE 1. PERCENTAGE OF SPERMATOZOA SHOWING FORWARD MOTION AFTER 6 HOURS STORAGE IN THE DIFFERENT MEDIA AT APPROXIMATELY 5° C (MEAN OF FIVE TRIALS)

Salt	pH of Solution	Molality of Solution				
		.04	.07	.10	.13	.16
Sodium citrate	6.5	17.8	26.6	*32.6*	26.6	9.6
	7.0	15.0	23.0	11.8	6.2	3.4
	7.5	20.2	28.2	4.0	1.8	0.2
Sodium phosphate	6.5	3.8	18.6	16.0	20.0	13.0
	7.0	4.0	*34.0*	*40.0*	*41.0*	7.4
	7.5	8.6	*48.0*	*34.0*	20.2	7.0
Potassium citrate	6.5	3.8	*32.0*	25.0	21.6	8.8
	7.0	3.6	20.4	13.8	7.0	8.0
	7.5	2.6	19.2	10.8	6.4	1.6
Potassium phosphate	6.5	1.6	2.8	13.2	4.4	1.4
	7.0	4.0	13.6	25.0	6.4	1.8
	7.5	10.0	28.6	*40.0*	14.4	5.0

the spermatozoa to become apparent but not so long that motility would be completely lost in many of them and the differences between diluents consequently obscured.

After the six hour period of refrigeration, examinations were made of the spermatozoa for estimating motility. The diluted samples were allowed to warm at room temperature for a minimum of 15 minutes and the vials were shaken thoroughly. A small drop was then placed on a slide and covered with a cover slip. Examinations were made at 450 X and the percentage of spermatozoa showing forward motion estimated from various fields to the nearest 5 percent except where motility was very low and finer steps of estimation were attempted. Semen samples were labelled only with code numbers in order that motility might be estimated without knowledge of the treatment that had been given; thus no particular order was followed in examining the samples. Examination required approximately one minute per sample.

Five different experimental trials were made of the effects of different

diluents on motility. Each trial consisted of a determination of the effects of 60 different diluents upon the spermatozoa from one composite collection. Fresh diluents were prepared for each trial thus sampling the technical error in the preparation of diluents as well as variation in spermatozoa and day-to-day environmental changes. The actual pH of the diluent including the spermatozoa was checked at the end of the refrigerating period in several instances and found to show little or no change indicating an adequate buffer capacity of the various diluents.

TABLE 2. MEAN SQUARES OF MOTILITY PERCENTAGES DUE TO THE HIGHER ORDER INTERACTIONS OF POSITIVE ION (P), NEGATIVE ION (N), MOLALITY (M), HYDROGEN ION CONCENTRATION (H) AND TRIAL (T)

Interaction	D.F.	Mean Square
P x N x H x M	8	9.6
P x N x H x M x T	32	56.3
P x N x H	2	131.3
P x N x H x T	8	45.4
P x N x M	4	555.9**
P x N x M x T	16	107.3
P x H x M	8	89.5*
P x H x M x T	32	31.2
N x H x M	8	200.2**
N x H x M x T	32	49.2

* probability of 0.05.
** probability of 0.01.

No attempt was made to determine the osmotic pressure of the different diluents. The permeability of the cell membrane of the spermatozoa is not known for the different kinds of ions under study at different temperatures and for the different molalities and hydrogen ion concentrations. Also, spermatozoa may not under all conditions require an isotonic solution to maintain maximum motility.

Results and Discussion

The average motility of the spermatozoa stored in the different diluents is shown in table 1.

Inspection of table 1 indicates that no one optimal level of either molality or pH applies to all four salts. The percentage values for motility were transformed to angles (angle = arc sin $\sqrt{\text{percentage}}$) as given by Bliss (1937). A test was made of the higher order interactions (table 2) and the significance of three of these: P x N x M, P x H x M, and

N x H x M bore out the indication. It consequently became necessary to find the optimal combination of molality and pH for each of these 4 salts. Applying Duncan's (1951) method, for the test of significance, the means for the diluents (8 in number) which were found significantly different from the rest and not significantly different from each other are shown in italics in table 1. Sodium phosphate showed the widest range in both pH and molality which would support high motility. In fact five of the eight high values were for this salt at pH of 7.0 and 7.5 and molalities of .07, .10 and .13. The apparent optimum pH and molality respectively for each salt selected for the "control" diluents in the fertility studies of the second experiment, were: sodium citrate, 6.5 and .10; sodium phosphate, 7.5 and .07; potassium citrate, 6.5 and .07; and potassium phosphate, 7.5 and .10. The three experimental diluents for each salt involved one of the following changes from the optimum: (1) increase in molality to .16, (2) decrease in molality to .04 and (3) change in pH to 7.5 in the case of the citrate buffers or to 6.5 for the phosphate buffers.

Experiment 2

Material and Methods

Female rabbits that had been isolated individually and injected intravenously with 50 i. u. of chorionic gonadotrophin to produce ovulation were inseminated artificially with 0.5 cc diluted semen. These females were killed 24 hours later and the eggs flushed from the oviducts with physiological saline and examined under the binocular microscope for evidence of fertilization (cleavage). Any female was discarded that showed old corpora lutea in her ovaries which might have been functional when she was inseminated. Also data were not used for any female from which fewer than 5 eggs were recovered. The rabbits were run in four trials of 24 females each. A trial included one acceptable test rabbit for each experimental diluent and three for each control diluent (one to balance each rabbit on experimental diluents for that salt) and any one trial was completed before the next was begun. The number of rabbits run at any one time varied with the number available (ranging from 6 to 12) but the order in which the experimental diluents were tested in each trial was set up at random. Retests were made at the next opportunity to take the place of any eliminated rabbits.

Semen was collected from the same bucks used in experiment 1 and by the same method. The different diluents used were added to mixed semen in the same way as in experiment 1, cooled to approximately 5° C. for six hours, then allowed to warm at room temperature for approxi-

mately 15 minutes and subjected to the microscopic test for determining the percentage of spermatozoa in forward motion. Samples were tested in a random sequence using the method of coding mentioned in experiment 1. As soon as the spermatozoa in a given diluent were checked for motility, they were used in inseminating a female rabbit.

Results and Discussion

The relative motilities of the spermatozoa observed in the second experiment were in satisfactory agreement with those observed in the first (tables 1 and 3).

TABLE 3. EFFECT OF CONTROL AND EXPERIMENTAL DILUENTS ON MOTILITY AND FERTILITY OF SPERMATOZOA

	Control diluent				Experimental diluent				Difference in average fertility from control per unit difference in av. motility
Salt	pH	Mol.	Percent semen motility	Percent egg fert.	pH	Mol.	Percent semen motility	Percent egg fert.	
Sodium citrate	6.5	.10	56	71	6.5	.16	11	48	.51
					6.5	.04	14	32	.93
					7.5	.10	10	34	.80
Sodium phosphate	7.5	.07	58	78	7.5	.16	5	53	.47
					7.5	.04	6	60	.35
					6.5	.07	18	62	.40
Potassium citrate	6.5	.07	40	50	6.5	.16	10	59	—.30
					6.5	.04	3	17	.89
					7.5	.07	22	31	1.06
Potassium phosphate	7.5	.10	40	61	7.5	.16	3	18	1.16
					7.5	.04	6	3	1.71
					6.5	.10	10	81	—.67

Fertility determinations for each control diluent were made on 12 rabbits and for each experimental diluent were made on 4 rabbits. The percentage of egg fertility is the unweighted mean of the percentages of the eggs recovered from each rabbit which were fertilized. Semen with better motility was available for the second experiment and greater sampling error might be expected for the higher motility percentages of the control than for the lower percentages of the experimental diluent. Varying a single factor, either pH or molality produced changes in motility for experimental study (table 3) ranging from 18 percent for potassium citrate (pH 7.5, 0.07 M) to 53 percent for sodium phosphate (pH 7.5, 0.16 M). At the same time some motility was preserved in all experimental diluents so that there was gross evidence of life and thus of potential fertilizing ability.

The preliminary analysis of the percentages of motile spermatozoa

and percentages of fertilized eggs (transformed to angles) included average correlations of motility and fertility between rabbits within the control and within the experimental groups. These values were 0.17 and 0.02, respectively, neither approaching statistical significance. The correlation of average motility and average fertility between the different experimental and respective control groups was .60 ($P.<0.05$). Thus for the particular 12 treatments involved there was a significant association induced between motility and fertility.

The differences in average percent of eggs fertilized for an experimental diluent from that for its control diluent ranged from an apparent decline of 58 percent for potassium phosphate (pH 7.5, 0.04 m.) to an apparent increase of 20 percent for the same salt (pH 6.5, 0.10 m.). Expressing these in terms of differences in average fertility from the control per unit difference in average motility gave a range from 1.71 in the former case to —.67 in the latter. The changes for 10 out of the 12 experimental diluents were positive, i.e., as motility was depressed so was fertility. This is a further indication of the tendency noted above for motility and fertility to be associated as a result of treatment. Whether or not the associations may be considered homogeneous for the different treatments requires statistical test. Ratios such as these differences in average fertility per unit difference in average motility do not appear to be normally distributed. A test was used which is believed to be unaffected by the distribution of the variable under study, viz. the "H" test based on Rank (Kruskal and Wallis, 1952). A ratio, $Re=(Fe-Fc)/(Me-Mc)$, was calculated for each experimental rabbit. The fertility for each experimental rabbit, Fe, minus the mean fertility for the 12 control rabbits on the same salt, Fc, was divided by the motility of the spermatozoa used on the experimental rabbit, Me, minus the mean motility of the spermatozoa used on the controls, Mc. Significant differences between treatment groups were shown in these ratios for the experimental rabbits (P of 0.05). Further examination of the treatment groups for each salt by the same test showed that those for potassium phosphate were the only ones which by themselves varied significantly. It is recognized, however, that the sampling error of both motility and fertility determinations in this experiment is high and the efficiency of the statistical test is not great.

Other actual differences may not have been shown significant but it is believed that the ones found at least demonstrate the lack of necessary dependence of fertility on motility as conditioned by environment. The results obtained are consistent with the hypothesis that fertility and motility of spermatozoa are two largely independent cellular phe-

nomena. It should be emphasized, however, that motility was studied *in vitro* and that the tissue fluids of the genital tract, their pH, temperature and their chemical and physical composition may change motility or reactive spermatozoa *in vivo* and a change in their fertilization capacity could ensue. The nature and importance of such factors affecting motility of spermatozoa inside the genital tract is not known at the present time. To whatever extent they exist, however, the problem of predicting fertility of spermatozoa from laboratory examinations of motility is made more complicated.

Summary

Motility and fertility variations of rabbit spermatozoa were induced by experimentally altering pH and osmotic pressure. Four different buffer systems (mono- and disodium phosphate, mono- and dipotassium phosphate, sodium citrate and citric acid and potassium citrate and citric acid) have been used as sperm diluents. The first study was designed to determine for each buffer system the optimum molality and pH for motility as well as hypertonic and hypotonic concentrations, and pH values which would support some motility but at a low level. In the second experiment these particular variations in pH and molality were studied to determine their effects on the relation between motility and fertility. The differences in fertility in the experimental diluents from the fertility in the control diluents per unit difference in motility were found to differ significantly from one experimental diluent to another. These results showed that motility *in vitro* and fertility are phenomena which may or may not be affected similarly by an environmental factor.

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ARSENICAL SUPPLEMENTS IN LAMB FATTENING RATIONS [1, 2, 3, 4]

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THE stimulatory effect of 3-nitro-4-hydroxyphenylarsonic acid on the growth of poultry was reported by Morehouse and Mayfield (1946), and Bird *et al.* (1949), confirmed these findings. Morehouse (1949) reported that this compound increases growth in turkey poults; and, Carpenter (1951) found that it stimulates growth in weanling pigs. He also reported (1951) increased weight gains and feed efficiency in lambs due to feeding a commercial mineral and vitamin supplement containing 3-nitro-4-hydroxyphenylarsonic acid.[6]

The experiments reported here were conducted to study further effects of each of three arsenical supplements fed to growing-fattening lambs.

Experiment 1

Procedure

Thirty-six black-faced western feeder lambs ranging in weight from 62 to 99 lb. were used in this experiment. All were dipped in Cooper's arsenical dip and drenched with three ounces of a 1 percent solution of copper sulphate and one ounce of phenothiazine one week before the experiment began.

The basal ration consisted of 50 percent ground alfalfa hay, 45 percent ground yellow corn, and 5 percent of 42 percent protein soybean oil meal by weight. Potassium arsenite, arsanilic acid, and arsonic acid were used as arsenic supplements. Because little work had been done with arsenicals in lamb rations the recommended levels for non-

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[6] This compound is referred to as arsonic acid throughout the remainder of this paper.

ruminant animals were used in this experiment. Arsonic acid was used at levels of 0.002, 0.004, and 0.006 percent of the total ration, and the other compounds were supplied at levels of equal arsenic content. The potassium arsenite was included because it is the active ingredient in Fowler's Solution which has long been used as a conditioner and stimulant, particularly for show animals. The arsenical compounds were premixed with the soybean oil meal before being mixed with the ground alfalfa hay and ground corn in a twin spiral mixer.

The lambs were randomized as to treatment, arsenic level, feeding position, and killing date. They were turned into their individual feeders from approximately 7:00 to 9:00 a.m. and 5:00 to 7:00 p.m. daily. Water and trace-mineralized salt were available at all times. Wood shavings were used for bedding. An area on the right rib section of the lambs was clipped to the skin at the beginning of the experiment so that wool grown during the experiment might be measured, using the method reported by Starks *et al.* (1953). The lambs were weighed at fourteen-day intervals.

To study the disappearance of arsenic from the tissues, twelve lambs were slaughtered on the eighty-fourth day of the experiment (one from each level of each arsenical and three from the basal ration), and all other lambs were put on the basal ration. Three days later twelve more lambs were slaughtered, and the remaining ten lambs (two basal lambs died) were slaughtered after being on the basal ration for six days.

Results and Discussion

The data were analyzed statistically, adjusting final weights for differences in initial weights and feed consumption. No significant difference was found (table 1).

Blood samples were taken and prothrombin times were determined by Quick's method. There was no significant difference between treatments (mean 19.9 seconds; standard deviation 2.34 seconds). Based on the work of Griminger *et al.* (1953), one would not expect the clotting time to be prolonged, as these rations contained 50 percent alfalfa hay which is a good source of Vitamin K.

Rumen samples were taken near the end of the experiment from two lambs on the highest level of each arsenical and two basal lambs. Dr. Lorraine Gall [7] found the rumen microflora to be normal and observed no environmental or bacteriological difference between treatments.

[7] Then at National Dairy Research Laboratories, Inc., Oakdale, Long Island, New York; presently on a Fulbright Fellowship at Wallaceville Animal Research Station, Wallaceville, New Zealand, c/o Dr. I. J. Cunningham.

Fecal samples taken on the eighty-second day of the experiment showed no difference between treatments in numbers of common parasite eggs.

Sieburth *et al.* (1951) reported reduced numbers of clostridia due to feeding antibiotics to pigs and turkey poults. Fecal samples were taken at the time of slaughter. No significant difference in clostridia numbers between treatments was found.

TABLE 1. RESULTS OF THREE ARSENICAL SUPPLEMENTS FED TO GROWING-FATTENING LAMBS FOR 84 DAYS

Ration	No. of Lambs	Average			
		Gain	Gain per Day	Feed Consumed	Feed per lb. Gain
		lb.	lb.	lb.	lb.
Basal	7	32.0	.381	256.4	7.93
Basal + K-Arsenite, %					
.002	3	30.3	.360	280.0	9.23
.004	3	29.3	.347	257.3	8.77
.006	3	35.7	.413	257.3	7.21
Basal + Arsanilic acid, %					
.002	3	28.0	.333	267.3	9.54
.004	3	26.6	.317	244.3	9.16
.006	3	28.0	.333	255.7	9.13
Basal + Arsonic acid, %					
.002	3	26.6	.337	249.0	9.33
.004	3	32.3	.368	247.0	7.64
.006	3	36.3	.420	292.3	8.05

Histological studies were made of livers, kidneys, and adrenal glands of all lambs. No difference was observed between treatments.

The livers, kidneys, a fat sample, and a muscle sample were saved from each lamb for chemical analysis for arsenic. Repeated use of the Kingsley-Schaffert (1951) arsenic determination failed to give satisfactory recovery of arsenic when known amounts of a working arsenic solution were added to the tissue samples. When the Kingsley-Schaffert method was modified (as suggested by Dr. R. J. Evans, Michigan Agricultural Experiment Station), by mixing tissue with a saturated solution of magnesium nitrate, drying, and then ashing in a muffle furnace at 600° F., much better recovery of arsenic was obtained. Further chemical analyses are being made on these tissues and on tissues from later experiments.

Two lambs on the basal ration died during the test. Cause of death was diagnosed as enterotoxemia by the diagnostic clinic of the College of Veterinary Medicine.

No symptom of arsenic toxicity was observed during the experiment on any level of any of the arsenicals fed. All lambs completing the experiment made good gains, ranging from 0.31 to 0.42 lb. per day. The general appearance of the entire group was very good. All 34 lambs slaughtered produced carcasses that were graded prime by a United States government meat grader. The dressing percentages ranged from 50 to 56 percent. Wool growth measurements showed no difference between treatments.

Experiment 2

Procedure

The second experiment was planned because the levels of arsenic supplementation in Experiment 1 had neither stimulated growth nor proven toxic. Higher levels of arsenicals were fed in an effort to reach a stimulatory level. The procedure followed was as in Experiment 1 with these exceptions: 60 lambs were used, all lambs were sheared before the experiment began, two different rations were used, the arsenical supplements were fed at higher levels, and the lambs were vaccinated for enterotoxemia. Arsonic acid was fed at levels of 0.006, 0.012, and 0.024 percent of the total ration, with the other two compounds used at levels to supply equivalent amounts of arsenic.

Ration 1 was the same as used in Experiment 1, while Ration 2 consisted of 18 percent ground alfalfa hay, 32 percent ground wheat straw, 45 percent ground yellow corn, and 5 percent of 42 percent soybean oil meal by weight. Thus Ration 1 had a crude protein content of approximately 15 percent compared with about 10 percent for Ration 2. This provided a test of the effect of arsenical supplements on a poorer quality ration in addition to the basal ration previously used.

Twelve lambs were put on each basal ration and two lambs on each of three levels of the three arsenical compounds.

Results and Discussion

The lambs were fed on the experimental rations for 63 days. Tissues were not saved for chemical analysis. The initial and final weights and feed consumed were analyzed statistically. No significant arsenic effect was found within the adequate protein ration. However, the lambs receiving arsenicals with the poor basal ration made gains significantly greater than the lambs on this basal ration alone (P less than 0.05). There was no significant difference between arsenic compounds or levels of arsenic. As would be expected, there was a highly significant difference

between the two rations. Lambs on Ration 1 ate more feed and made faster gains than the lambs on Ration 2. Individual lamb gains ranged from 0.27 to 0.60 lb. per day. All lambs ate well and made good gains. Table 2 gives the gains and feed consumption for lambs completing the experiment.

TABLE 2. RESULTS OF THREE ARSENICAL SUPPLEMENTS FED TO GROWING-FATTENING LAMBS FOR 63 DAYS

Ration	No. of Lambs	Average Gain	Average Gain per Day	Average Feed Consumed	Average Feed per lb. Gain
		lb.	lb.	lb.	lb.
Basal No. 1	11[1]	30.3	.481	226.7	7.48
Basal No. 2	12	18.5	.294	164.0	8.86
Ration 1					
Basal + K-arsenite, %					
.006	2	26.5	.421	186.5	7.04
.012	1[2]	24.0	.381	188.0	7.83
.024	2	27.0	.428	187.0	6.93
Basal + arsanilic acid, %					
.006	2	24.0	.381	218.5	9.10
.012	2	35.5	.563	211.5	5.95
.024	2	37.0	.587	219.5	5.93
Basal + arsonic acid, %					
.006	2	25.5	.405	171.5	6.72
.012	2	25.0	.397	226.0	9.04
.024	2	30.0	.476	242.5	8.08
		Av.	.449	205.7	7.40
Ration 2					
Basal + K-arsenite, %					
.006	2	21.0	.333	158.0	7.52
.012	2	23.5	.373	184.0	7.83
.024	2	28.5	.452	191.0	6.70
Basal + arsanilic acid, %					
.006	2	28.0	.444	183.5	6.55
.012	2	21.5	.341	193.5	9.00
.024	2	24.0	.381	173.0	7.20
Basal + arsonic acid, %					
.006	2	20.5	.325	212.5	10.36
.012	2	24.0	.381	219.5	9.14
.024	2	22.5	.357	174.0	7.73
		Av.	.377	187.7	8.00

[1] Removed from experiment because of infected tooth.
[2] Removed from experiment because of shearing cut.

Rumen samples, studied by Dr. Gall, from one lamb on the highest level of each compound on both rations and one lamb from each basal ration showed no apparent difference in rumen flora between different treatments. Findings were normal for sheep eating this type of ration.

There was no significant difference between treatments in the prothrombin time (mean 31.5 seconds; standard deviation 4.05 seconds).

Parasite egg counts in fecal samples from lambs on different treatments gave some indication that the higher levels of arsenic had reduced the number of coccidia. Other parasite eggs were very few in all lambs.

No clinical symptom of toxicity was observed at any time. Carpenter (1951) reported that arsonic acid fed to pigs at levels of 0.01 and 0.02 percent of the ration was toxic, the arsenic accumulating more rapidly in the liver and kidney. A species difference in susceptibility to arsenic damage is indicated. Wool growth measurements showed no difference between treatments (table 2).

Experiment 3

Procedure

This experiment was planned to study further the indications in Experiment 2 that arsenic stimulated growth on the poorer quality ration and that the higher levels of the arsenicals reduced the number of coccidia. The general plan was the same as in Experiment 2 with these exceptions: 64 lambs were used, all lambs were self-fed, 44 percent soybean oil meal was used, arsonic acid at the level of 0.024 percent of the ration was the only arsenic supplement fed, and the lambs were not vaccinated for enterotoxemia. The lambs were grouped into eight lots of eight lambs each, two lots on each basal ration and two lots of each basal plus 0.024 percent arsonic acid. The rations consisted of 50 percent roughage and 50 percent concentrates by weight for the first three weeks; then the mixture was changed to 60 percent concentrates and 40 percent roughage.

Fecal samples were taken at the beginning of the experiment and after four weeks to determine the effects of the arsonic acid on the intestinal parasites.

Results and Discussion

The lambs were on the experimental rations for 63 days. Analysis of initial and final weights and feed consumed showed no significant arsenic effect in either ration (table 3).

The differences in average daily gain between treatments was offset by differences in the feed consumed. There was no evidence in this test that the arsenic improved the rate of gain on the poor quality ration as had been indicated by Experiment 2. The weight gains of individual lambs on Ration 1 ranged from 0.33 to 0.73 lb. per day and on Ration 2 from 0.16 to 0.52 lb. per day.

Counts were made for coccidian oocysts, and for trichostrongyloid, and strongyloides eggs at the beginning of the trial and again at the end

TABLE 3. ARSONIC ACID AT 0.024 PERCENT LEVEL FED TO GROWING-FATTENING LAMBS FOR 63 DAYS

Ration	Pen No.	No. of Lambs	Average Initial Weight	Average Daily Gain	Average Feed Consumed per Day	Average Feed per lb. Gain
			lb.	lb.	lb.	lb.
Basal No. 1	1	8	70.6	.46	3.3	7.05
	2	8	70.9	.48	3.5	7.36
Basal No. 2	3	8	70.5	.38	3.6	9.44
	4	8	70.3	.37	3.5	9.42
Basal No. 1 + Arsonic acid	5	8	72.1	.54	3.9	7.27
	6	8	72.6	.54	4.0	7.38
Basal No. 2 + Arsonic acid	7	8	70.3	.30	3.2	11.10
	8	8	74.3	.38	3.5	9.25

of four weeks. There was no evidence that the arsenic affected the number of any of these parasite eggs (all counts were relatively low).

All lambs completed the experiment and no toxicity symptom was observed. The eyes of a number of lambs appeared abnormally red, but when examined carefully no difference was found between basal and arsenic supplemented lambs. It was thought that dust from the ground roughage caused this eye irritation.

Blood studies of these lambs showed no significant difference between treatments in sedimentation rate, hemoglobin, hematocrit, number of red cells, or number of white cells.

No difficulty was encountered with enterotoxemia. In a few cases there was severe scouring for several days. These cases cleared up after drenching again with one ounce of phenothiazine.

Toxicity Studies

Since no toxicity symptoms had been observed on any of the levels of the arsenicals fed, and some of these levels have been high in terms of swine and poultry tolerance, an experiment is in progress to determine the toxic level of these arsenicals in rations for fattening lambs. This study, which will be reported later, indicates that arsonic acid is less toxic to lambs than arsanilic acid and potassium arsenite on the basis of weight gains, feed consumption, and toxicity symptoms. Histological studies and analyses of arsenic content of tissues are being made. The lambs on 0.2 and 0.4 percent levels of arsanilic acid became convulsive before the feeding period ended at 56 days. This does not follow the same order of toxicity reported by Frost (1953) for ducks where arsanilic acid was less toxic than arsonic acid. Possibly this indicates species differences.

Summary

Individual full feeding and group self-feeding were used to determine the effect of three different arsenical compounds on weight gains and wool production of growing-fattening lambs. Practical type Corn Belt growing-fattening rations made up of alfalfa, corn, and soybean oil meal were used in the trials.

Potassium arsenite, arsanilic acid, and arsonic acid were used to supply equal amounts of arsenic at levels of 0.002, 0.004, 0.006, 0.012, and 0.024 percent of the total ration based on the arsonic acid. None of these arsenicals fed at these levels consistently increased weight gains significantly when fed with an excellent quality or a poor quality ration. Experiment 2 indicated an increase in weight gains, which Experiment 3 did not, due to the arsenic on the poor quality ration. The gains of the lambs on the excellent ration exceeded those of the lambs on the poor ration, the difference being significant at the 1 percent level in both trials in which these were compared.

In Experiment 3 arsonic acid at the 0.024 percent level did not reduce the numbers of coccidia as Experiment 2 had indicated it might.

Rumen microflora, prothrombin time, and wool growth were not observed to be affected by any of the treatments.

No histological difference was observed in the livers, kidneys, and adrenal glands from the lambs in the first experiment.

No toxicity effect was observed at any time during the first three experiments. All rations were eaten satisfactorily and all lambs made very good gains.

These results do not indicate any benefit from including these arsenicals in a practical farm ration for growing-fattening lambs. It appears that these arsenicals are not as toxic for sheep as they are for swine and poultry.

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UTILIZATION OF INTRAVENOUSLY-ADMINISTERED CAROTENE BY SHEEP AND CATTLE[1]

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CATTLE normally circulate a relatively large quantity of beta-carotene in the blood stream, and substantial amounts of the pigment may be present in liver and kidney tissues and in the fat deposits. Sheep, goats, and swine, however, circulate no appreciable quantities of carotene in the blood. Kon and Thompson (1951) have summarized their work and that of others which showed that conversion of orally administered beta-carotene to vitamin A occurs in the intestinal wall.

Recent reports are conflicting concerning the availability to the animal of parenterally administered carotene as a precursor of vitamin A. In studies with vitamin A deficient lambs, Klosterman *et al.* (1949) found that colloidal carotene injected into the blood stream was rapidly removed with no increase in blood or liver vitamin A. Warner and Maynard (1952) reported that intravenously injected carotene in coconut oil would not cure vitamin A deficient calves, but that similar injections of aqueous colloidal suspensions of carotene significantly increased blood levels of vitamin A. Eaton *et al.* (1951) reported a very limited conversion of carotene to vitamin A in the blood of vitamin A deficient dairy calves given intravenous injections of aqueous suspensions of carotene. With pigs, Hentges *et al.* (1952) found that water miscible preparations of carotene administered intravenously or intramuscularly resulted in complete remission of vitamin A deficiency symptoms. Recently, Bieri and Pollard (1953) have demonstrated rapid conversion of carotene to vitamin A in rats, using intravenous injections of carotene dispersed in water with polyoxythylene 20 sorbitan monopalmitate (Tween 40).

It was the purpose of the experiments reported here to study the conversion to vitamin A of aqueous carotene preparations administered intravenously to sheep and beef calves.

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Experimental Procedure

Eight yearling, fine-wool wethers ranging in weight from 85 to 100 lb. were fed a low carotene ration containing milo, cottonseed meal, wheat straw, and a mineral mixture for 8 weeks. At the end of this period blood plasma vitamin A values varied from 10 to 28 mcg./100 ml. At this time an aqueous carotene [4] preparation dispersed with Tween 40 was injected intravenously. This preparation was made by dissolving the carotene in a minimum amount of chloroform, adding the dispersing agent and finally adding water with constant stirring. This procedure gave a perfectly clear solution fully miscible with water and blood plasma (Bieri, 1951). One wether, No. 7, received approximately 14.0

TABLE 1. VITAMIN A AND CAROTENE CONTENT OF LIVER AND BLOOD OF VITAMIN A DEFICIENT HEREFORD CALVES, AND OF LIVER AFTER INTRAVENOUS ADMINISTRATION OF CAROTENE

	Prior to Injection				Following Injection	
	Blood Plasma (mcg./100 ml.)		Liver Tissue (mcg./gm. dry matter)		Liver Tissue (mcg./gm. dry matter)	
Calf No.	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	Carotene
24	2.7	8.1	6.0	1.5	3.8	2.2
31	7.2	8.3	3.2	0.9	1.4	2.1
33	6.5	10.1	1.2	0.0	4.5	3.7
34	4.9	8.1	1.2	0.6	1.6	1.8
44	4.3	9.9	1.8	0.8	0.0	3.6
51	6.9	13.2	1.8	1.0	4.1	1.2
57	4.9	3.6	2.4	0.0	6.3	2.9
Mean	5.3	8.7	2.5	0.7	3.1	2.5

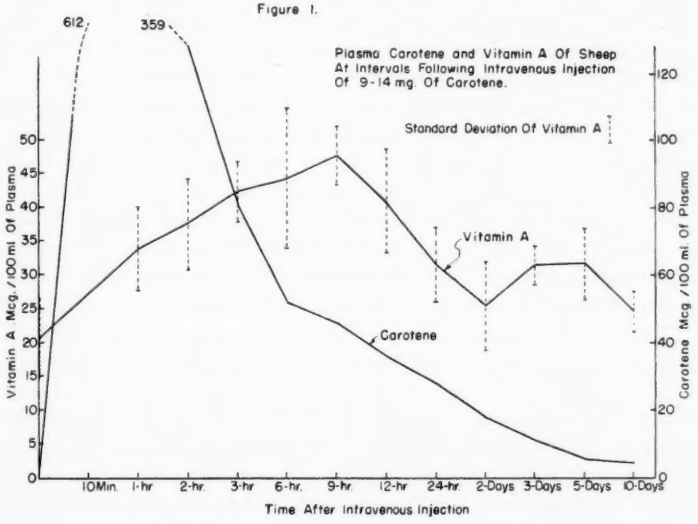
mg. of carotene, and the others 9.2 mg. each, approximately 7 times the minimum daily requirement. Blood samples were taken by venous puncture before injection and at the following intervals thereafter: 10 minutes, 1, 2, 3, 6, 9, 12, and 24 hours, and 2, 3, 5, and 10 days. The samples were collected in citrated tubes and the plasma analyzed for carotene and vitamin A by the method of Kimble (1939).

Seven 4-month-old Hereford calves weighing between 150 and 200 lb. were given treatment similar to that of the wethers. These calves were from dams which had been fed a low-carotene ration similar to that described above, for several months. Liver carotene and vitamin A levels

[4] Crystalline carotene was kindly supplied by Barnett Laboratories, Long Beach, California.

at 3 months of age were very low (table 1). Several of the calves, particularly 33, 44, and 51, exhibited typical vitamin A deficiency symptoms before the beginning of the experiment, i.e., diarrhea, muscular incoordination, and partial blindness in one or both eyes. Calf 34 received approximately 17.0 mg. of carotene, calf 24 about 14.3 mg., and all others 13.7 mg. each, approximately 7 times the minimum daily requirement. Liver biopsy samples were taken three days following injection by the method described by Whitehair *et al.* (1952). The liver samples were frozen until analyzed for vitamin A and carotene

Figure 1.



according to the method of Gallup and Hoefer (1946). Data obtained from these experiments were analyzed statistically according to Snedecor (1946).

Results and Discussion

Mean values of the plasma carotene and vitamin A of the wethers are presented graphically in figure 1. The initial plasma carotene was very low, 1.4 mcg. per 100 ml. From a mean of 612 mcg. 10 minutes following injection, the plasma carotene fell rapidly during the first 6 hours to 52 mcg., and thereafter continued to decrease in a steady and

consistent manner. Five days after injection, practically all carotene had disappeared from the blood stream.

The initial mean value of plasma vitamin A in the sheep was 21.4 mcg. per 100 ml. Within 1 hour the level had risen to 34 mcg. and continued to rise until 9 hours after injection when the mean value was 47.9 mcg., or more than twice the initial value. Thereafter the plasma vitamin A declined slightly until at 10 days it was 24.7 mcg. Differences in plasma vitamin A between initial samples and later samples were highly significant ($P=.01$) when analyzed statistically. Values for samples taken 10 minutes after injection are not included in figure 1 because of spuriously high values for vitamin A. That this apparent conversion was not due to an artifact because of an effect of the dispersing agent was checked by injecting an equivalent amount of Tween 40 into two wethers with normal vitamin A stores and blood levels. No increase in plasma vitamin A resulted from such treatment.

Wether no. 7, the first sheep treated, received a larger dose than the others. Since analysis of this blood had been conducted before the others were treated, and the apparent conversion of carotene to vitamin A noted, it was decided to reduce slightly the amount of carotene administered. However, this difference in dosage did not result in any major difference in vitamin A content of the plasma.

Liver samples obtained by sacrificing the sheep approximately 12 days after injection were analyzed for carotene and vitamin A. Carotene values ranged from 0.0 to 4.0 mcg. per gm. of dry matter, with a mean of 3.1 mcg., compared to a mean of 1.8 for eight similar wethers not given carotene (negative controls). The difference between means for controls and treated wethers was not statistically significant. Liver vitamin A values were highly variable. Since this may have been due to improper handling of the samples prior to analysis, the data are not reported.

The mean carotene content of the plasma of the calves at the start of the experiment was 11.9 mcg. per 100 ml. (figure 2). Ten minutes after injection of the carotene it had increased to 307 mcg.; thereafter, it fell rapidly until at 2 hours it was 89 mcg. This level was maintained for about 24 hours, and then began to drop slowly. Ten days following injection the mean was still 32 mcg.

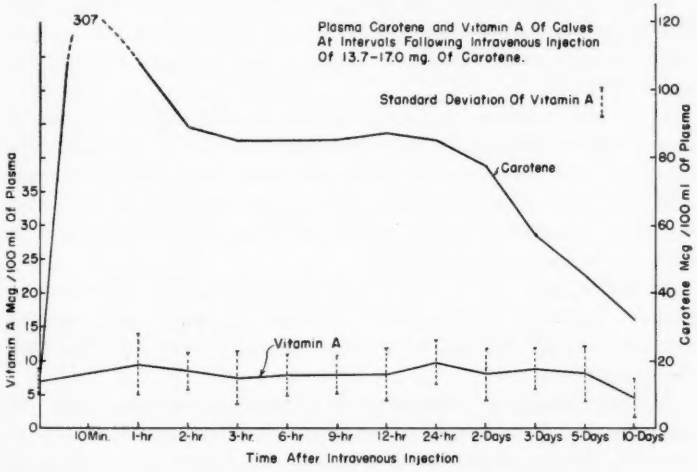
Individual variations of plasma vitamin A were such that there were no significant differences between means of samples taken at different times after injection, in contrast to the results of the sheep experiment.

Mean plasma vitamin A values were lower at 10 days following injection than at the start of the experiment. These data provided no evidence of a significant conversion of carotene to vitamin A.

Liver samples taken from the calves 3 days following injection were found to contain only small amounts of carotene or vitamin A (table 1). These low values were corroborated by the progressive development of clinical symptoms of vitamin A deficiency in the calves. The liver vitamin A values did not reflect any appreciable conversion of carotene.

Clinical symptoms of vitamin A deficiency did not diminish during the course of the 10-day experimental period in any of the treated

Figure 2



calves. The state of depletion of calves 33, 44, and especially 51, appeared to become more advanced during the 10-day experimental period.

The results of the experiment with sheep are contrary to those reported by Klosterman *et al.* (1949). It is believed that the nature of the dispersion of carotene is the basis for these differences, and that, in common with the rat and pig, sheep can readily convert intravenously-administered carotene to vitamin A. The results obtained with Hereford calves are contrary to the work of Eaton *et al.* (1951), and to that of Warner and Maynard (1952), who found that dairy calves were able

to convert aqueous suspensions of carotene to vitamin A. Further investigation will be required to determine whether or not the apparent difference in carotene metabolism between the beef and dairy breeds is real.

It has been suggested that animals with carotene normally circulating in the blood are less efficient converters (in the intestinal wall) than are animals without circulating carotene. While this may be true, the data from this study suggest that in certain species carotene may be converted to vitamin A in sites other than the intestinal wall.

Summary

Eight fine-wool wethers and seven Hereford calves were given single intravenous injections of a solublized, aqueous carotene preparation. Blood samples were collected at intervals and analyzed for carotene and vitamin A.

In wethers, increases in plasma vitamin A after carotene injections were highly significant, the mean values at 3, 6, 9, and 12 hours after injection being approximately twice the initial value. Blood carotene, after an initial rise, fell rapidly during the first 6 hours following injection, then declined slowly for the remaining 10 days. The effect on liver stores was questionable.

In calves, carotene injection caused no significant differences in plasma vitamin A values. Plasma carotene remained quite constant near 85 mcg. per 100 ml. for 2 to 24 hours following injection, then decreased slowly

No liver storage of vitamin A or carotene was evident from the comparison of liver samples taken 1 month before and 3 days after injection. Advanced vitamin A deficiency symptoms present in some of the calves were not relieved and appeared to progress during the 10 days of the experiment.

The data obtained in these experiments indicate a wide difference between cattle and sheep in their ability to convert intravenously injected carotene to vitamin A.

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THE INFLUENCE OF DIETHYLSTILBESTROL ON CERTAIN BLOOD AND LIVER CONSTITUENTS OF LAMBS [1]

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DIETHYLSTILBESTROL, when implanted into growing-fattening lambs, increased the rate of gain and feed efficiency and decreased the carcass quality as reported by Andrews *et al.* (1949), Jordan (1950 and 1953), O'Mary *et al.* (1952), and Means *et al.* (1953). Digestibility trials by Jordan and Bell (1952), Whitehair *et al.* (1953), and Jordan (1953) have shown that diethylstilbestrol treatment has little or no influence on feed digestibility. The increased rate of gain and feed efficiency thus cannot be explained on the basis of increased digestibility of feed. The explanation of these phenomena may be (1) the more efficient utilization of nutrients after absorption (2) a change in the composition of the increased weight gains. Indirect evidence exists for both of these explanations. The reports of Andrews *et al.* (1949), Jordan (1950 and 1953), O'Mary *et al.* (1952), and Means *et al.* (1953) indicated an altered fat metabolism in treated animals. These workers reported a decreased deposition of external fat following estrogen treatment. Jordan (1950 and 1952), O'Mary *et al.* (1952), and Means *et al.* (1953) indicated that treated animals produced soft, watery carcasses but the relation between increase in carcass water content and increased weight gain needs further study. The investigations reported herewith were an attempt to determine the influence of diethylstilbestrol on metabolism insofar as it could be deduced from its effects on certain blood and liver constituents of lambs.

Materials and Methods

Blood Studies

Twenty crossbred western wether feeder lambs which averaged 75.2 lb. each were assigned at random to two lots. One lot was treated with 15 mg. of diethylstilbestrol implanted subcutaneously in the ear; the

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other lot was not treated and served as a control. The animals were fed *ad libitum* a ration consisting of 50 percent ground yellow corn and 50 percent chopped alfalfa-bromegrass hay. The treated lambs at the time of bleeding had gained an average of 0.33 lb. per day compared to 0.24 lb. per day for the controls. Blood samples were taken after 23 days on experiment.

The bleeding procedure allowed estimation in the statistical analysis of the effect of the time of bleeding in relation to the time of feeding. One-half of the animals were bled between 7:30 and 10:00 a.m. The lambs were selected for bleeding entirely at random and a treated and a control animal were bled alternately. The remaining lambs were bled similarly between 1:30 and 3:30 p.m. The a.m. blood samples, for practical purposes, represent fasting or preabsorptive blood since the last feed had been given to the animals the previous afternoon. Feed was given to the lambs about 8:00 a.m. on the day of bleeding but little or none of the feed was consumed before the completion of the a.m. bleeding. The p.m. blood samples can be considered as postabsorptive blood. Approximately 40 to 45 ml. of blood from each lamb were collected in a 50 ml. centrifuge tube containing 0.5 ml. of saturated potassium oxalate. The blood, after mixing gently by inversion, was placed immediately into a thermos jug containing ice.

In the laboratory, the blood was again mixed gently and two Van Allen hematocrit tubes [3] were filled from each sample. Hematocrit determinations were made as described by Van Allen (1925). Four ml. of whole blood were withdrawn and deproteinized according to the procedure of Folin and Wu (1919). The protein-free filtrate was stored in a refrigerator until glucose and non-protein nitrogen could be determined. These tests were made within 24 hours. The remainder of the blood was centrifuged to obtain the plasma which was kept frozen until analyzed.

Glucose was determined by the method of Durham *et al.* (1950) using anthrone reagent. The method was modified to include heating in a boiling water bath as recommended by Green and Wade (1952). Nonprotein nitrogen was determined according to the method of Koch and McMeekin (1924). The suggestions of Thompson and Morrison (1951) were followed with regard to the use of Nessler's reagent. The plasma proteins were determined according to the procedure given by Hawk *et al.* (1947). Plasma lipids were determined according to Boyd (1938).

[3] Kimble No. 46745, Kimble Glass, Toledo 1, Ohio.

Liver Studies

Liver samples from two treated and two control lambs receiving each of five different rations were obtained at the time of slaughter. Those lambs on the basal ration had been used previously for the blood studies. The lambs were slaughtered after 76 days on experiment.

Approximately 50 to 75 gm. of tissue were cut from about the same place on each liver, wrapped in freezer locker paper, and placed into a thermos jug containing dry ice. The samples were frozen almost immediately and were kept frozen until thawed before weighing for analysis.

Dry matter was determined by drying in duplicate a small sample at 100 degrees C. overnight. Results of all the liver analyses were calculated on the dry weight basis. Glycogen was determined by the indirect method of Seifter *et al.* (1950). Nitrogen was determined by micro-Kjeldahl and protein was calculated as N $\times$ 6.25. Lipids were determined by the method of Boyd (1938) after extraction had been carried out according to Bloor (1942).

Results and Discussion

Blood Constituents

Table 1 gives the data for those blood constituents which were significantly affected either by estrogen treatment, time of bleeding, or by both. Table 2 shows the results of the statistical analysis of the data. The analysis of variance was made as described by Snedecor (1946). When a significant interaction of estrogen treatment and time of bleeding was obtained, further analyses were made to compare the estrogen treatments within each respective time of bleeding and to compare times of bleeding within each respective estrogen treatment.

Table 2 shows that the estrogen treatment in these lambs had a significant effect over both times of bleeding on hematocrit, plasma fibrinogen, and plasma free cholesterol.

Table 1 shows that the treated animals had lower hematocrits at both times of bleeding but the difference between treated and control lambs was greatest in the p.m. samples. This hematocrit effect agrees with the results obtained by Dinusson *et al.* (1950) with steers, Sturkie (1951) with chickens, Steinglass *et al.* (1941) with rats, and Witten and Bradbury (1951) with humans. These workers found a decreased red blood cell count and/or hematocrit in estrogen treated animals. Roberts *et al.* (1944) reported no influence of estrogen on hematocrit

TABLE 1. EFFECT OF DIETHYLSTILBESTROL ON CERTAIN BLOOD CONSTITUENTS (MEAN VALUES)

Treatment	No. of Lambs	Time of Sampling	Hematocrit %	N.P.N. mg. %	Fibrinogen gm. %	Globulin gm. %	Total Protein gm. %	Phospho-lipid mg. %	Free Cholesterol mg. %	Total Lipid mg. %
	5	A.M.	35	30.5	0.44	2.55	6.07	19.4	12.1	164
Estrogen Implanted	5	P.M.	30	25.1	0.48	2.63	6.12	27.8	14.7	174
	10	Both	32.5	27.8	0.46	2.59	6.10	23.6	13.4	169
	5	A.M.	37	29.4	0.29	2.97	6.33	13.3	11.3	159
Control	5	P.M.	37	33.3	0.30	2.02	5.42	36.5	11.4	171
	10	Both	37	31.4	0.30	2.50	5.88	24.9	11.4	165

in dogs. The increased percentage of plasma in treated animals could influence the analytical results of those blood constituents which are calculated in terms of quantity per 100 ml. of plasma. This, however, cannot account for the significant estrogen effects found in this investigation since both fibrinogen and free cholesterol were higher, rather than lower, in treated animals as compared to their controls.

TABLE 2. RESULTS OF THE ANALYSIS OF VARIANCE OF THE BLOOD DATA

Source of Variation	Degrees of Freedom	Constituents Showing a Significant Effect
Between estrogen treatments over both times of bleeding	1	Hematocrit,* Fibrinogen,** Free Cholesterol*
Between times of bleeding over both estrogen treatments	1	Total Lipid*
Estrogen treatments × times of bleeding interaction	1	N.P.N.,* Globulin,* Total Protein,* Phospholipid**
Within lots	16	
Between estrogen treatments within A.M. samples	1	None
Between estrogen treatments within P.M. samples	1	N.P.N.,* Globulin,* Total Protein,* Phospholipid*
Between times of bleeding within control animals	1	Globulin,** Total Protein,** Phospholipid**
Between times of bleeding within treated animals	1	Phospholipid*

* Significant at the 5% level.
** Significant at the 1% level.

The values for fibrinogen were higher for treated lambs than for controls. The physiological significance of this effect is not known. Thayer *et al.* (1945) and Stamler and Warner (1951) report increased blood clotting time in estrogen treated chickens. Stamler and Warner found, however, that the fibrinogen content of the plasma was normal in these birds.

The free cholesterol content of the plasma of estrogen treated lambs was higher than that of the control lambs. This effect cannot be explained. The administration of estrogen to chickens has been shown by Fleischmann and Fried (1945) to increase the total cholesterol of the blood plasma. Dinusson *et al.* (1950) found, however, that the

plasma cholesterol of diethylstilbestrol treated beef heifers tended to be lower.

Total lipid was significantly affected by the time of bleeding. In both treated and control lambs, the total lipid content of the plasma was higher in the p.m. blood samples. This might be explained on the basis of the nutrients which had been absorbed from the morning feed. There was a significant interaction of estrogen treatment and time of bleeding for non-protein nitrogen, globulin, total protein, and phospholipid.

There was no significant difference between treated and control animals in plasma non-protein nitrogen in the a.m. samples but a significantly lower quantity was found in the treated lambs in the p.m. blood samples.

Globulin and total protein remained constant over both times of bleeding in the plasma of treated lambs but the level in the control animals was significantly lower in the p.m. blood samples.

Both the ability to maintain plasma protein levels and the decreased plasma N.P.N. indicated a more efficient utilization of absorbed nitrogen by the treated animals in this experiment. These effects might be related to the increased nitrogen retention observed in treated lambs by Jordan and Bell (1952), Whitehair *et al.* (1953), and Jordan (1953).

Table 1 shows that treated and control lambs had more phospholipid in the p.m. blood samples but the increase over the a.m. level was smaller for the treated lambs. There was no statistically significant difference between the phospholipid content of the plasma of treated and control animals in the a.m. samples but the treated lambs had significantly less plasma phospholipid in the p.m. blood samples. The physiological significance of this effect is unknown.

The statistical analyses indicated no significant effect of estrogen treatment or time of bleeding on the plasma content of glucose, albumin, ester cholesterol, total cholesterol, or neutral fat. The mean values for these constituents over both estrogen treatments and both times of bleeding were 70 mg. percent glucose, 3.07 gm. percent albumin, 30.2 mg. percent ester cholesterol, 42.6 mg. percent total cholesterol, and 80 mg. percent neutral fat. The coefficients of variation for these values respectively are 14.3, 9.1, 12.0, 10.3, and 16.2.

Liver Constituents

Table 3 gives data for the total dry matter and ester cholesterol in the liver. Table 4 gives the results of the statistical analysis of these

TABLE 3. EFFECT OF DIETHYLSTILBESTROL ON CERTAIN LIVER CONSTITUENTS (MEAN VALUES)

Ration	No. of Lambs	Treatment	Total Dry Matter lb.	Ester Cholesterol %
Basal	2	Control	0.38	0.41
	2	Treated	0.52	0.27
Basal plus protein	2	Control	0.43	0.56
	2	Treated	0.50	0.40
Basal plus trace minerals	2	Control	0.38	0.56
	2	Treated	0.46	0.42
Basal plus molasses	2	Control	0.47	0.38
	2	Treated	0.44	0.22
Basal plus protein, trace minerals, and molasses	2	Control	0.46	0.56
	2	Treated	0.51	0.32

data. All liver constituents were calculated as percentage of the dry liver sample weight and the values were transformed for analysis into arc sin of the square root of the percentage. The analysis of variance was then made in the usual manner (Snedecor, 1946).

There was a significant difference for total liver dry matter and ester cholesterol between treated and control lambs. While there was no significant difference between treated and control lambs in the percentage of dry matter, this value multiplied by the wet liver weight resulted in a greater amount of total liver dry matter present in the

TABLE 4. RESULTS OF THE ANALYSIS OF VARIANCE OF THE LIVER DATA

Source of Variation	Degrees of Freedom	Constituents Showing a Significant Effect
Between estrogen treatments	1	Total Liver Dry Matter,** Ester Cholesterol*
Between rations	4	None
Estrogen treatments × rations	4	None
Within lots	10	

* Significant at the 5% level.
** Significant at the 1% level.

treated animals. This was true for all rations except when molasses was added to the basal. The increase in liver dry matter was probably due to hypertrophy which resulted from the extra work of detoxification of the implanted diethylstilbestrol. Zondek *et al.* (1943) by *in vitro* studies, found that estrone was inactivated by liver much more easily than diethylstilbestrol. Within a specified time, about twice as much liver was required to inactivate a given amount of diethylstilbestrol as for an equivalent amount of estrone.

The decreased level of liver ester cholesterol, like the increased plasma free cholesterol in treated lambs, is difficult to explain. There is a possibility that the two are related, i.e., the ester cholesterol of the liver may contribute to raise the free cholesterol in the plasma but this is merely speculation. Had the liver samples been obtained by biopsy at the time of bleeding, such a relationship perhaps could have been established.

The statistical analysis indicated no significant effect of estrogen treatment on the liver content of dry matter (percentage), glycogen, protein, phospholipid, free cholesterol, total cholesterol, neutral fat, or total lipid. The mean values for these constituents over both estrogen treatments and over the five rations were 29.44 percent dry matter, 0.23 percent glycogen, 13.76 percent protein, 12.23 percent phospholipid, 0.76 percent free cholesterol, 1.17 percent total cholesterol, 7.8 percent neutral fat, and 21.5 percent total lipid. The coefficients of variation for these values respectively are 1.65, 28.6, 12.4, 3.14, 7.05, 7.78, 10.7, and 3.79.

There was neither a significant effect of ration nor a significant interaction of ration and estrogen treatment for any of the liver constituents studied.

Information will be published later on the detailed composition of the rations and the effect of the rations on growth, fattening, and certain carcass characteristics of the lambs.

Summary

Experiments were conducted to determine the influence of diethylstilbestrol implantation on blood and liver composition in feeder lambs. Blood analyses were performed on samples taken from the jugular vein of 20 lambs on the 23rd day of the experiment. Fifteen mg. of diethylstilbestrol were implanted subcutaneously in the ear of one-half of the animals; the other half was not treated and served as controls. The bleeding procedure involved taking samples at random from one-

half of the treated and one-half of the control lambs in the a.m. and similarly from the remainder in the p.m.

The estrogen treated lambs had lower hematocrit, and higher levels of plasma free cholesterol and fibrinogen than their controls. Total plasma lipid was higher in the p.m. samples for both treated and control lambs. There was an interaction of estrogen treatment and time of bleeding for non-protein nitrogen, globulin, total protein, and phospholipid. There were no statistically significant differences between treated and control animals for these constituents in the a.m. blood samples. In the p.m. blood samples, the treated animals had a significantly lower quantity of non-protein nitrogen and phospholipid and a significantly higher quantity of globulin and total protein than their controls. There were no significant effects of estrogen treatment or time of bleeding on plasma glucose, albumin, ester cholesterol, total cholesterol, or neutral fat.

Liver samples from two estrogen treated and two control lambs receiving each of five different rations were obtained at the time of slaughter. A significantly greater amount of total liver dry matter and a significantly smaller amount of liver ester cholesterol was found in the treated lambs. Estrogen treatment did not affect the liver content of percentage dry matter, glycogen, protein, phospholipid, free cholesterol, total cholesterol, neutral fat, or total lipid. Ration had no effect on the liver constituents studied.

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THE EFFECT OF CERTAIN MINERAL SUPPLEMENTS ON LAMBS INFECTED WITH THE STOMACH WORM (*HAEMONCHUS CONTORTUS*) [1]

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PREVIOUS work has shown that the feeding of trace mineralized salt and steamed bone meal in the ration enabled lambs to better withstand stomach worm (*Haemonchus contortus*) infection (Weir *et al.* 1948; Badr 1949; Richard *et al.* 1954). The present experiments were conducted to determine the mineral(s) involved in the alleviation of the effects of stomach worm infection on lambs.

Experimental Procedure

The data reported are from two consecutive experiments. The experimental procedure was essentially the same for both experiments. Parasite-free lambs were produced from ewes fed the rations shown in table 1 (experiment I) and table 3 (experiment II). The ewes were allotted at random and placed on their respective rations about 6 weeks prior to lambing. Progesterone was used to synchronize the estrous cycle of the ewes in order to minimize age differences among the lambs (O'Mary *et al.* 1950).

The lambs were full-fed rations corresponding to those received by their dams, with the exception of oat straw. Oat straw was omitted from the lamb ration. The other ration ingredients were fed *ad libitum* to the lambs. Prior to infection, precautions were taken to keep the lambs as free from parasites as possible. Qualitative determinations of the eggs in the feces showed the pre-experimental parasite infection of the lambs in all lots to be negligible. When approximately 3 months of age, the lambs in each lot, with the exception of control animals, were each given 50,000 stomach worm larvae. The larvae were administered in water by means of a drenching syringe.

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In experiment II the infective larvae were from two different sources. One-half of the lambs which were infected in each lot were given larvae from stomach worms reared for several successive generations in sheep fed trace mineralized salt. The remaining lambs were infected

TABLE 1. RATIONS FED EXPERIMENT I EWES AND LAMBS
Lb. per Day

Lot No.		Before Lambing	After Lambing
I	Basal		
	Mixed grass-legume hay	2.0	2.5
	Oat straw[4]	2.0	2.0
	Ground corn	.75	1.2
	Iodized salt	Free choice	Free choice
II	Basal + cobalt		
	Mixed grass-legume hay	2.0	2.5
	Oat straw[4]	2.0	2.0
	Ground corn	.75	1.2
	Cobaltized salt[1]	Free choice	Free choice
III	Basal + manganese		
	Mixed grass-legume hay	2.0	2.5
	Oat straw[4]	2.0	2.0
	Ground corn	.75	1.2
	Salt + manganese[2]	Free choice	Free choice
IV	Basal + trace minerals		
	Mixed grass-legume hay	2.0	2.5
	Oat straw[4]	2.0	2.0
	Ground corn	.75	1.2
	Trace mineralized salt[3]	Free choice	Free choice

[1] Cobaltized salt contained 6.72 gm. $CoSO_4$ in 100 lb. salt.
[2] Salt + manganese contained 100.50 gm. $MnSO_4 \cdot H_2O$ in 100 lb. salt.
[3] Trace mineralized salt contained 5.00 gm. $CoCO_3$, 50.25 gm. $CuSO_4$, 50.25 gm. Fe_2O_3, 100.5 gm. $MnSO_4 \cdot H_2O$, and 361.8 gm. $FeSO_4$ in 100 lb. salt.
[4] Oat straw was omitted from the lamb ration. The other ration ingredients were fed *ad libitum*.

with a low mineral strain of larvae from stomach worms whose hosts received only iodized salt.

Non-infected lambs were used as controls for each ration. The control of the experiment against possible reinfections was checked through periodic examination of the fecal material of control animals to which stomach worm larvae had not been administered.

Data were collected on weight gains, blood hemoglobin levels, fecal egg counts, feed consumption, plasma calcium, plasma niacin, and the vitamin B_{12} content of blood and rumen ingesta. Hemoglobin was

determined by the oxyhemoglobin method. Nicotinic acid was determined by the microbiological method of U. S. Pharmacopoeia (1947). Plasma calcium was determined by the method of Clark and Collip (1925). Vitamin B_{12} was determined by the microbiological method of

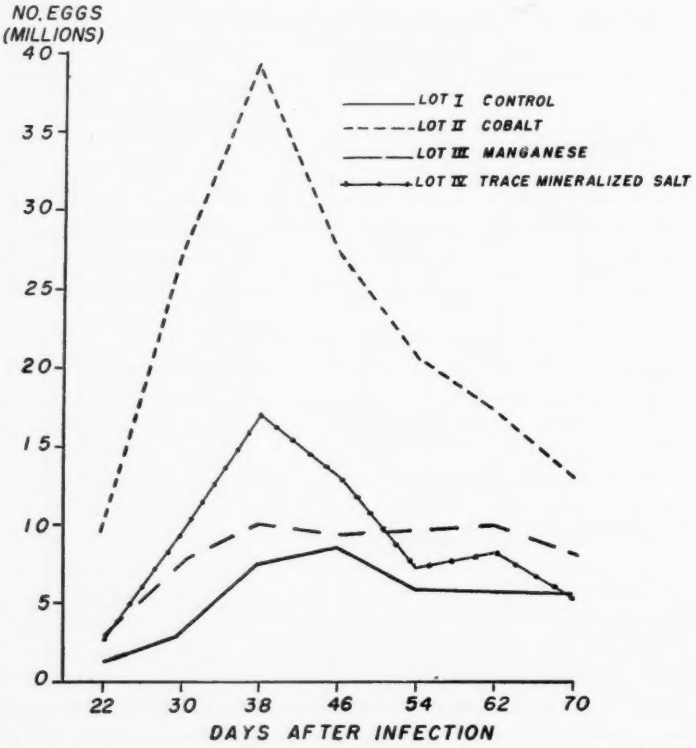

Figure 1. Average fecal egg counts of Experiment I lambs infected with 50,000 larvae.

Thompson *et al.* (1950). Fecal egg counts were determined by the method of Stoll (1930). Data were analyzed according to standard statistical procedure (Snedecor, 1948).

Results and Discussion

Experiment I

The average numbers of stomach worm eggs passed in the feces of the experiment I lambs are shown in figure 1. A marked increase in

egg counts was noted in the feces of the cobalt fed lambs. After the initiation of egg production, the number of eggs in the feces of the cobalt supplemented lambs increased markedly, attaining a peak of 40 million stomach worm eggs daily per lamb. In comparison with the lambs which did not receive added cobalt, reference to their respective infection peaks shows that the combined egg production of five lambs on the basal ration scarcely equalled the eggs passed by one of the cobalt supplemented lambs. Further, the cobalt supplemented lambs, at the peak of the infection, passed approximately four times as many eggs as the lambs which received manganese as the only supplement to the basal ration. Egg production was higher when lambs received cobalt alone than when the complete trace mineral mixture, including cobalt, was fed. Weir *et al.* (1948) noted that supplementation of the ration of the host with trace minerals and steamed bone meal increased the number of worm eggs in the feces. This was confirmed by Badr (1949) and Richard *et al.* (1954). The present findings show that iron, copper, and manganese were without appreciable effect, but that cobalt directly or indirectly increased the fecundity of the female worm.

In order to correlate fecal egg counts with the actual number of stomach worms present in the host animal, certain lambs in each lot were sacrificed at 33 and 73 days following infection. The lambs were autopsied and worm counts determined. The data revealed no significant differences in the worm counts among lots. This would seem to lend credence to the supposition that cobalt stimulates the production of eggs by the stomach worm rather than causing a larger number of worms to develop from a given number of larvae.

Anemia was a characteristic symptom of severe stomach worm infection. The first apparent decline in the blood hemoglobin of the infected lambs was noted 11 days after infection. The most severe anemia developed in the lambs which received cobalt, with or without the other trace minerals. The severe anemia of the cobalt fed lambs was accompanied by a high rate of mortality (60%) as shown in table 2. Recovery did not ensue when the concentration of hemoglobin in the blood decreased below 3 grams percent.

The highest average blood hemoglobin levels during the entire period of stomach worm infection were maintained by the lambs which received no mineral supplementation. Also, there were no death losses from infection when the basal ration without minerals was fed. Although one death did occur on the manganese supplemented ration, it appears that manganese supplementation had no appreciable effect

upon the post-infection decline in blood hemoglobin. With the exception of the lamb which succumbed to the infection, the anemia was no more severe when manganese was fed than when the basal ration was unsupplemented (P greater than 0.05).

During the 72-day period following infection, the basal fed lambs gained an average of 0.20 lb. daily (table 2). In contrast there was an average daily loss of 0.63 and 0.39 lb. respectively when cobalt and trace minerals (Fe, Cu, Co, Mn) were fed. The magnitude of the

TABLE 2. GROWTH AND MORTALITY SUMMARY OF INFECTED AND CONTROL LAMBS

Experiment I

Supplement..	Lot I None		Lot II Co		Lot III Mn		Lot IV Fe, Cu, Co, Mn	
Treatment...	Control	Infected	Control	Infected	Control	Infected	Control	Infected
No. lambs	2	6	2	5	2	5	2	5
Infection period (days)	....	72	....	72	....	72	....	72
Av. initial wt. (lb.)	58.5	62.0	57.5	57.0	53.5	68.0	55.0	55.8
Av. final wt. (lb.) [1]	78.0	76.8	80.5	11.8	75.0	63.4	80.0	27.4
Av. daily wt. change (lb.)	+0.27	+0.20	+0.32	−0.63	+0.30	−0.06	+0.35	−0.39
Lamb deaths	0	0	0	3	0	1	0	3
Mortality (percent)	0	0	0	60	0	20	0	60

[1] Av. final wt. of infected lambs $= \frac{\text{final wt of surviving lambs}}{\text{No. lambs infected}}$

weight losses in the cobalt and mineral supplemented lots was due largely to the high mortality. Likewise, the average daily loss in weight of the lambs which received manganese was due to the death which occurred on that ration. The four manganese supplemented lambs which survived the infection showed a positive growth response of 0.15 lb. per lamb daily.

All rations appeared to be adequate for normal growth of the non-infected control lambs. The basal ration exhibited no superiority upon growth over the cobalt and mineral supplemented rations when the lambs were not infected with stomach worms. The same was true of the manganese supplemented ration.

It appears from these data that the feeding of trace mineralized or cobaltized salt with the basal ration was of greater benefit to the parasite than to the host animal and that supplementation of the lamb ration with either was detrimental when the lambs were exposed to stomach

worm infection. This seems to be a reversal of previous findings. In this experiment, however, steamed bone meal was not fed with the trace minerals tested, which indicates that calcium and phosphorus also may be critical dietary factors in increasing the resistance of lambs to stomach worm infection.

TABLE 3. RATIONS FED EXPERIMENT II EWES AND LAMBS
Lb. per Day

Lot No.		Before Lambing	After Lambing
I	Basal		
	Mixed grass-legume hay[1]	3.4	3.4
	Oat straw[3]	.71	.86
	Ground corn	1.0	1.25
	Iodized salt	Free choice	Free choice
II	Basal + cobalt		
	Mixed grass-legume hay[1]	3.35	3.35
	Oat straw[3]	.73	.88
	Ground corn	1.0	1.25
	Cobaltized salt[2]	Free choice	Free choice
III	Basal + cobalt + steamed bone meal		
	Mixed grass-legume hay[1]	3.35	3.35
	Oat straw[3]	.73	.88
	Ground corn with 1% steamed bone meal	1.0	1.25
	Cobaltized salt[2]	Free choice	Free choice
IV	Basal + steamed bone meal		
	Mixed grass-legume hay[1]	3.35	3.35
	Oat straw[3]	.73	.88
	Ground corn with 1% steamed bone meal	1.0	1.25
	Iodized salt	Free choice	Free choice

[1] Mixed grass-legume hay contained 0.09 p.p.m. of elemental cobalt.
[2] Cobaltized salt contained 6.72 gm. $CoSO_4$ in 100 lb. salt.
[3] Oat straw was omitted from the lamb ration. The other ration ingredients were fed *ad libitum*.

Experiment II

Experiment II was conducted to determine the effect of steamed bone meal (Ca and P) plus cobalt on lambs infected with stomach worms and to study further the role of minerals in the alleviation of symptoms of stomach worm infection in lambs. The rations used are shown in table 3.

The stimulatory effect of cobalt on stomach worm egg production was further demonstrated by the average fecal egg counts of the experiment

II lambs presented in figure 2. Stomach worm eggs were detected in the feces of all lambs 19 days after infection. The lambs showed evidence of a progressively increasing infection as indicated by the steadily

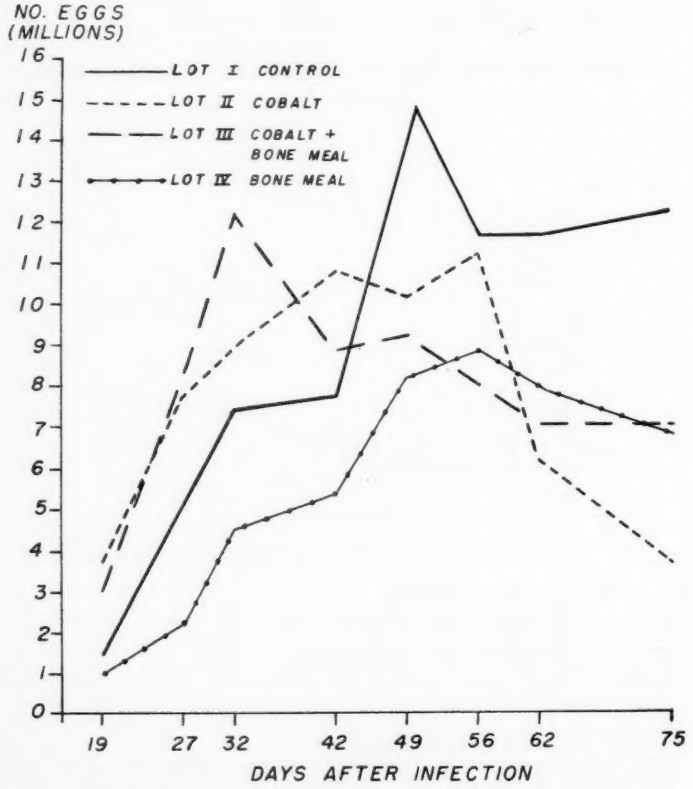

Figure 2. Average fecal egg counts of Experiment II lambs infected with 50,000 larvae.

rising egg production. Egg production, after reaching a peak, declined. The initial increase in egg production was more rapid in lambs which received cobalt or cobalt plus steamed bone meal than in lambs which received the basal ration or bone meal, and the infection peak occurred several days earlier in the cobalt supplemented lots. The lambs which

received steamed bone meal only passed the smallest numbers of stomach worm eggs consistently.

Data on blood hemoglobin levels (table 4) show that all infected lambs became anemic. The lambs which received cobalt plus steamed bone meal maintained the highest blood hemoglobin levels. Cobalt alone exerted a marked protective effect against the development of anemia. The most severe anemia and the highest mortality rate was observed in lambs fed the basal ration. The anemia was quite severe in lambs which received steamed bone meal only; however, bone meal

TABLE 4. THE CONCENTRATION OF HEMOGLOBIN IN THE BLOOD OF EXPERIMENT II LAMBS

Gm. per 100 ml.

Lot No.	Supplement	No. lambs	Treatment[1]	Days After Infection						
				0	18	38	52	60	70	76
I	None	2	Control	12.97	10.11	10.33	9.06[2]	9.96	10.04	10.15
		10	Infected	12.16	7.41	4.79	4.42(2)[3]	4.95(1)	4.55	4.51(3)
II	Co	5	Control	13.16	10.89	10.30	10.62	11.04	11.15	11.23
		9	Infected	11.72	8.73	7.06	6.22	6.46	7.62(1)	7.85
III	Co+ bone meal	6	Control	12.48	11.15	11.19	11.19	11.18	11.41	11.55
		12	Infected	12.28	9.26	7.79	7.46	8.19	8.89	9.91
IV	Bone meal	2	Control	12.29	10.05	10.89	9.94	10.42	10.66	10.73
		8	Infected	12.53	8.84	5.38	3.54	3.79	3.73	3.78

[1] Infected lambs were given 50,000 stomach worm larvae.
[2] One control lamb in lot I died of pneumonia.
[3] ( ) = No. of lamb deaths from stomach worm infection.

seemed to increase somewhat the resistance of lambs to stomach worm infection since there were no death losses on that ration.

Death losses resulting from infection are shown in tables 4 and 5. Sixty percent of the lambs which received the basal ration died of haemonchiasis. The mortality was considerably lower in the cobalt supplemented lots. There were no death losses among the lambs which received steamed bone meal plus cobalt or steamed bone meal alone. The previous nutritional history of the parasite had no effect on the severity of the infection.

The weights of the infected lambs are shown in table 5. Rations supplemented with steamed bone meal and cobalt produced the best weight gains. Gains were less when cobalt was fed without steamed bone meal but still considerably better than those obtained with the basal ration or the basal ration plus bone meal. The addition of cobalt to the basal ration enabled lambs to gain slightly during the period of infection.

Supplementation with steamed bone meal alone seemed to produce an increased resistance to infection but failed to evoke a positive growth response. However, when cobalt and steamed bone meal were fed in combination, the weight gains of the infected lambs were almost equal to those of the non-infected control lambs receiving the same ration.

All lambs in lots I and IV, including the non-infected control lambs, made poor weight gains. In contrast, the control lambs in lots II and III, which received supplemental cobalt, made good weight gains during

TABLE 5. GROWTH AND MORTALITY SUMMARY OF INFECTED AND CONTROL LAMBS

Experiment II

Supplement..	Lot I None		Lot II Co		Lot III Co + bone meal		Lot IV Bone meal	
Treatment...	Control	Infected	Control	Infected	Control	Infected	Control	Infected
No. lambs	2	10	5	9	6	12	2	8
Infection period (days)	....	76	....	76	....	76	....	76
Av. initial wt. (lb.)	58.5	49.5	68.4	52.1	54.8	50.2	75.0	56.3
Av. final wt. (lb.) [1]	53.0	24.4	93.8	56.7	80.3	73.9	75.0	52.0
Av. daily wt. change (lb.)	−0.07	−0.33	+0.33	+0.06	+0.33	+0.31	0	−0.06
Lamb deaths	1[2]	6	0	1	0	0	0	0
Mortality of infected lambs (percent)	....	60	....	11	....	0	....	0

[1] Av. final wt. of infected lambs $= \frac{\text{final wt. of surviving lambs}}{\text{No. lambs infected}}$

[2] One control lamb in lot I died of pneumonia.

the entire experimental period. This seems to indicate that the cobalt intake of the lambs in lots I and IV was inadequate for normal growth. The hay and corn used in the experiment were analyzed for cobalt by the method of Kidson and Askew (1940) and were found to contain 0.09 p.p.m. and 0.02 p.p.m. respectively. According to McNaught (1938) the minimum requirement of cobalt for sheep is 0.07 p.p.m. of the total ration. Since the proportion of hay to grain consumed was approximately 2:1, the lambs in lots I and IV theoretically received sufficient cobalt to meet the minimum requirement.

Control and infected animals within each lot were divided at the time of infection so that separate feeding records could be maintained. Feed consumption of infected lambs was highest in the case of lambs which received cobalt plus steamed bone meal. Lambs fed this ration

consumed about 28 percent more grain than lambs supplemented with cobalt only and approximately 150 percent more grain than lambs which received the basal ration or the basal ration plus steamed bone meal. This indicates that with adequate nutrition, lambs were able to maintain their appetites and well-being despite being heavily infected with stomach worms.

It was also observed that the lambs which received no added cobalt consumed approximately twice as much hay as grain. In the case of the cobalt supplemented lambs, the reverse was true.

TABLE 6. THE VITAMIN B_{12} CONTENT OF BLOOD AND RUMEN INGESTA

				Days After Infection		
				0	51	76
Lot No.	Supplement	No. Lambs	Treatment	Blood mγ/cc.	Blood mγ/cc.	Rumen Ingesta γ/gm.
I	Basal	2	Control	1.57	0.67	....
		4	Infected	2.33	0.57	26.2
II	Basal + Co	2	Control	3.07	3.52	....
		4	Infected	3.55	1.46	85.6
III	Basal + Co + bone meal	2	Control	3.81	2.43	....
		4	Infected	3.49	2.18	94.3
IV	Basal + bone meal	2	Control	2.09	0.74	....
		4	Infected	1.76	0.50	19.1

Vitamin B_{12} analyses were made on two control lambs together with four randomly selected infected lambs in each lot (table 6). Cobalt supplementation markedly increased the synthesis of vitamin B_{12} in the rumen. An increased level of B_{12} in the blood as a result of the increased synthesis is indicated. Stomach worm infection lowered the concentration of B_{12} in the blood, regardless of ration fed. The reduction was comparatively slight in lambs which received both cobalt and bone meal.

The level of nicotinic acid in the blood was approximately the same for all lambs indicating that supplementation with cobalt and bone meal, alone or in combination, did not increase the rate of synthesis of this vitamin in the rumen.

All calcium values were within the normal range. Plasma calcium remained constant without regard to ration fed or the severity of the infection.

At the time of slaughter, data were collected on selling price, carcass

grade, and dressing percentage. The uniformity and high quality of the lambs which received cobalt plus steamed bone meal were demonstrated by the carcass grades. Five of the 12 carcasses from infected lambs in that lot graded prime; six graded choice; one graded good. The carcasses of the lambs which received cobalt but no bone meal were of lower average quality. Two graded prime; four graded choice; one graded utility; one graded cull. In lot IV all of the lambs survived the stomach worm infection; however, the carcass grades attested to the poor condition of the bone meal fed lambs at the termination of the experiment. One graded good; six graded utility; one graded cull.

The average return per infected lamb was $6.15 in the lot which received the basal ration only; $11.53 in the lot where bone meal was fed in addition to the basal ration; $14.86 in the lot where the basal ration was supplemented with cobalt; and $20.86 in the lot where the basal ration was supplemented with bone meal and cobalt.

Summary

Experiments were conducted to determine the effect of mineral supplementation on lambs infected with stomach worms (*Haemonchus contortus*). The results show that the greatest resistance of lambs to infection was obtained by feeding cobalt and steamed bone meal in combination. The effect produced by cobalt supplementation may be due, at least in part, to an increased rate of synthesis of vitamin B_{12} in the rumen and its increased availability to the host. The exact mechanism by which steamed bone meal functions to increase resistance to stomach worm infection is not clear. It does appear, however, that when lambs are subjected to the severe strain of heavy parasitic infection, the requirements for factors contained in steamed bone meal are considerably increased.

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EFFECT OF SOMATOTROPIN ON MILK YIELD OF EWES [1]

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THE growth hormone isolated from bovine anterior pituitary glands has been shown by Donkers and Petersen (1953) to stimulate milk secretion of dairy cows in declining stages of lactation by about 10 percent. In an earlier trial these workers (1951) reported that cows receiving two multiple (injections made in two sites at the same time so as to expedite absorption) intramuscular injections of Somatotropin showed an average increase of 36.4 percent in milk yield during a 4-day period. Intramuscular injections were more effective than subcutaneous injections and multiple injections were more effective than single injections. These workers reported no change in fat percentage of the milk produced. Chung *et al.* (1953) injected six cows with 100 mg. of Somatotropin for six consecutive days and obtained a 50 percent increase in yield of milk and butterfat and 20 percent increase in blood glucose. Two trials were conducted at this station to determine the effect of Somatotropin on lactating ewes.

Procedure

In the first trial 12 ewes in their declining stages of lactation were allotted randomly into two groups of six ewes each. Data on milk yields, fat content and solids-not-fat were available on the ewes for nine weeks prior to treatment. The treated group of ewes received daily intramuscular injections of 25 mg. of Somatotropin in 5 cc. of saline solution while the control group was injected daily with the same amount of saline solution. The injections were made for six consecutive days and on the seventh day the ewes were hand milked (one-half of the udder). The lambs were removed 12 hours prior to milking. The ewes were milked on the third, fifth and tenth day after terminating the injections to determine the duration of effect of treatment. Standard methods of determining solids-not-fat were used and a modification of the Babcock test was used to determine fat content.

In this trial as well as the second trial 10 units of oxytocin were injected into the jugular vein of all ewes one to two minutes before

[1] Acknowledgment is made to Armour's Veterinary Research Laboratory through the courtesy of Dr. Irby Bunding for providing the Somatotropin used in this experiment.

[2] A portion of a thesis submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Animal Husbandry.

milking to facilitate evacuation of the mammary gland. The volume of milk obtained was measured to the nearest 5 ml. Sixty ml. were retained for fat and non-fat tests.

In the second trial four ewes, one each of the Columbia, Hampshire, Southdown and Rambouillet breeds, six to eight weeks post-partum were individually-fed a full feed of chopped alfalfa and ½ lb. hulless oats per head daily. A two-week adjustment period was used to acquaint the ewes and their lambs with the feeding and management conditions. The ewes and lambs were weighed twice weekly, and the ewes milked at each weighing. The lambs were allowed to run with the ewes at all times except on the days when the ewes were to be milked. On those days the lambs were removed 12 hours before milking the ewes. The milk was removed from one-half of the udder with a milking machine adapted for use on sheep. The ewes were first milked on August 27, again on August 31, September 3, and September 7 to determine their average milk yield prior to Somatotropin treatment. Each ewe received a daily intramuscular injection of 25 mg. of Somatotropin in 5 cc. of physiological saline from September 7 through September 27. The ewes were milked semi-weekly during this period. The ewes were milked on October 1 and October 5 to determine the duration of the effects of Somatotropin on milk yield and fat test. Somatotropin treatment was commenced again on October 5 and continued daily until October 11. On October 11 the ewes were milked again to determine their response to Somatotropin treatment after a seven-day interruption of the treatment. The response to treatment was measured as the change in milk yield and fat percentage using the pre-injection period for comparison.

Results

The results of the first trial are presented in table 1.

While the ewes to be treated were selected at random, milk yields of the control ewes during the four milkings prior to the treatment period were significantly greater than the milk yield of the ewes that were later treated. Conversely milk yield of the treated ewes during the four milkings post-treatment was materially greater than of the control ewes. This difference was highly significant.

Milk yield of the six control ewes declined during the four milkings post-treatment whereas the six ewes treated with Somatotropin produced about 35 percent more milk at the first milking following treatment than the milking prior to treatment.

The fat content and solids-not-fat of the six control ewes were some-

what variable but on the average increased slightly in comparison with previous milkings. The results for the six ewes receiving Somatotropin indicate a highly significant increase in fat percentage but a non-significant decrease in solids-not-fat in the milk at the first milking following treatment with a gradual return to approximately the pre-treatment level. Of the six ewes treated with growth hormone only one

TABLE 1. EFFECT OF SOMATOTROPIN ON LACTATION OF EWES

	Control, 6 Ewes			Treated, 6 Ewes		
	Av. Milk Yield, ml.	Av. Fat %	Av. Solids-Not-Fat %	Av. Milk Yield, ml.	Av. Fat %	Av. Solids-Not-Fat %
Dates Milked						
6/25	332	9.0	11.6	312	7.9	11.7
7/2	321	9.0	11.5	307	8.1	11.4
7/9	338	9.6	11.6	303	8.8	12.0
7/20	238	10.7	11.7	236	9.4	11.9
Av. Pre-treatment	307**[2]	9.6	11.6	290	8.6	11.8
7/26[1]	203	10.7	12.3	346**[3]	10.9**[3]	11.2
7/28	221	11.0	11.8	313	10.4	11.6
7/30	226	10.6	11.8	308	9.8	11.7
8/4	179	11.3	11.6	236	9.6	11.7
Av. Post-treatment	207	10.9	11.9	301**[4]	10.2	11.6

** Highly significant difference (P less than 0.01).

[1] Somatotropin injected daily 7/20 through 7/25. First post-treatment milking was 7/26.

[2] The control ewes produced a greater amount of milk during the pretreatment period than the ewes designated as treated during the same period.

[3] The treated ewes' milk production and fat test from the first milking following treatment was greater than from the last milking prior to treatment.

[4] Milk yield but not fat test of the treated ewes was greater than the control ewes during the post-treatment period.

failed to show an increase in fat content and a decrease in solids-not-fat. This ewe showed the smallest increase in milk yield. The six control ewes continued to go down in milk yield whereas the average milk yield of the six ewes treated with growth hormone remained above their yield prior to treatment. No adverse physiological effects were noted on the treated ewes and they appeared normal in every respect.

The effect of Somatotropin on milk yield and fat test in the second trial is shown in table 2.

A sizable increase in milk yields above what was obtained prior to

Somatotropin treatment occurred in three of the four ewes at the first milking after three days of treatment. The increase occurred in all four ewes after seven days of treatment. Average pretreatment milk yields were: 216, 329, 281, and 268 ml. for the Columbia, Hampshire, Southdown and Rambouillet ewes, respectively. During the period of Somatotropin treatment the average milk yields were: 300, 461, 366,

TABLE 2. EFFECT OF SOMATOTROPIN ON LACTATING EWES

Period	Milk Yield ml.	Fat Test %	Milk Yield ml.	Fat Test %
	Southdown Ewe		Rambouillet Ewe	
Pretreatment Av. 4 milkings	281	9.9	268	8.0
Treatment[1] Av. 6 milkings	366**	12.5**	374**	10.2**
Post-treatment[2] Av. 2 milkings	230	10.6	282	8.4
Treatment[3] 1 milking	390	11.2	380	9.0
	Columbia Ewe		Hampshire Ewe	
Pretreatment Av. 4 milkings	216	11.2	329	7.7
Treatment[1] Av. 6 milkings	300**	13.4**	461**	10.4**
Post-treatment[2] Av. 2 milkings	183	13.2	340	8.5
Treatment[3] 1 milking	170	15.8	460	10.0

[1] Somatotropin injected daily 9/7 through 9/27.
[2] No Somatotropin treatment from 9/28 through 10/4.
[3] Somatotropin injected 10/5 through 10/10.
** Highly significant difference.

and 374 ml., respectively, for the Columbia, Hampshire, Southdown and Rambouillet ewes. The difference in milk yield between the two periods was highly significant for all four ewes. Milk fat test increased during the treatment period from 1.5 to 3.5 percentage units with all four ewes showing an increase. The difference in the milk fat test before and during the treatment period was highly significant. During the seven-day period following treatment the milk yield and fat test declined to near the level previous to treatment. When Somatotropin treatment was resumed after seven days of no treatment, milk yield increased materially in three of the four ewes.

Feed Consumption

There was considerable variation in the amount of feed consumed by the four ewes in the different periods. The Southdown ewe consumed slightly less feed and the other three ewes consumed from 7 to 28 percent more feed during the treatment period than during the pretreatment period.

Ewe and Lamb Weights

The beginning ewe weights and weights at the close of the experiment were not materially different. The Rambouillet and Hampshire ewes weighed slightly more at the end of the treatment period than at the beginning of the trial. Weight of the Columbia ewe on the last day of treatment was only three pounds less than at the start of the experiment and the Southdown ewe weighed the same at the end of the experiment as at the beginning. All of the lambs made greater daily gains during the period in which the ewes were being treated. However, the number of animals and the length of time involved in this experiment are such that definite conclusions cannot be drawn relative to ewe or lamb gains.

Summary

The effect of daily intramuscular injections with 25 mg. of Somatotropin in 5 cc. of physiological saline in lactating ewes was studied. In the first trial the treated ewes produced about 40 percent more milk following the treatment period than the control ewes. A highly significant increase in fat test and a non-significant decrease in non-fat solids occurred following the treatment period.

In the second trial milk yield increased approximately 22 percent during the period when Somatotropin was injected. The fat test increased an average of 2.3 percentage units during the 21 days of Somatotropin treatment. The increase in both milk yield and fat test during the treatment period was highly significant. In both trials milk yield and fat test returned to near pretreatment levels three to ten days after terminating treatment. Feed consumption during the treatment period increased in three of the four ewes.

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NEWS AND NOTES

The forty-sixth annual meeting of the American Society of Animal Production will be held in the Hotel Sherman in Chicago, Illinois on November 26 and 27 with a dinner for the honored guest on November 28.

R. M. Jordan of the Animal Husbandry Department, South Dakota State College, who has been in charge of sheep research at that station has resigned his position and will take up new duties in the Animal Husbandry Department, University of Minnesota.

Livestock Production by Peters and Grummer, new second edition, was published by McGraw-Hill Book Co. Inc., New York, in June, 1954. The most recent findings in the feeding, breeding, and disease and parasite control of livestock have been included and all statistics have been brought up to date.

T. W. Dowe, Assistant Professor of the Animal Husbandry Department, University of Nebraska, received his Doctor of Philosophy degree May 23 from Kansas State College.

The second edition of *Raising Livestock* by Deyoe, Ross and Peters was published by McGraw-Hill Book Co., Inc., New York. This edition incorporates recent developments in such phases of livestock production as selecting, improving, feeding and maintaining health.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, doubled-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Cnarts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts". *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest concerning the members, and other items of interest to the membership. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. L. E. Casida, Department of Genetics, University of Wisconsin, Madison, Wisconsin, or to the publishers: Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. W. M. Beeson, Department of Animal Husbandry, Purdue University, Lafayette, Ind., or to the publishers, Boyd Printing Company, 372–374 Broadway, Albany 7, New York.